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Variation in movement patterns of red drum (*Sciaenops ocellatus*) inferred from conventional tagging and ultrasonic telemetry

Nathan M. Bacheler (contact author)[1]
Lee M. Paramore[2]
Summer M. Burdick[1]
Jeffrey A. Buckel[1]
Joseph E. Hightower[3]

Email address for contact author: bachelen@uwgb.edu

[1] Center for Marine Sciences and Technology
Department of Biology
North Carolina State University
303 College Circle Drive
Morehead City, North Carolina 28557
Present address for contact author: University of Wisconsin - Green Bay
Natural and Applied Sciences EN 317
2420 Nicolet Drive, Green Bay, Wisconsin 54311

[2] North Carolina Division of Marine Fisheries
Post Office Box 539
604 Harbor Road
Wanchese, North Carolina 27981

[3] United States Geological Survey
North Carolina Cooperative Fish and Wildlife Research Unit
Department of Biology
North Carolina State University
Raleigh, North Carolina 27695

Abstract—We used 25 years of conventional tagging data (n=6173 recoveries) and 3 years of ultrasonic telemetry data (n=105 transmitters deployed) to examine movement rates and directional preferences of four age classes of red drum (*Sciaenops ocellatus*) in estuarine and coastal waters of North Carolina. Movement rates of conventionally tagged red drum were dependent on the age, region, and season of tagging. Age-1 and age-2 red drum tagged along the coast generally moved along the coast, whereas fish tagged in oligohaline waters far from the coast were primarily recovered in coastal regions in fall months. Adult (age-4+) red drum moved from overwintering grounds on the continental shelf through inlets into Pamlico Sound in spring and summer months and departed in fall. Few tagged red drum were recovered in adjacent states (0.6% of all recoveries); however, some adult red drum migrated seasonally from overwintering grounds in coastal North Carolina northward to Virginia in spring, returning in fall. Age-2 transmitter-tracked red drum displayed seasonal emigration from a small tributary, but upstream and downstream movements within the tributary were correlated with fluctuating salinity regimes and not season. Large-scale conventional tagging and ultrasonic telemetry programs can provide valuable insights into the complex movement patterns of estuarine fish.

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Recent advances in conventional tagging and ultrasonic telemetry methods have substantially increased our understanding of the ecology of myriad estuarine organisms (e.g., Able and Hales, 1997; Able et al. 2005). For red drum (*Sciaenops ocellatus*), a long-lived estuarine and coastal fish species found along the Gulf and Atlantic coasts, conventional tagging and ultrasonic telemetry have been an invaluable tool to understand mortality rates and habitat use. For instance, fishery returns of conventional tags and the tracking of red drum tagged with ultrasonic transmitters have provided important information about the magnitude and seasonal patterns of fishery harvests and natural deaths (Latour et al., 2001; Bacheler et al., 2008a; Bacheler et al., 2009a). Telemetry has also been used to show that habitat-use patterns of subadult (i.e., ages 1–3) red drum are influenced by both abiotic and biotic factors (Dresser and Kneib, 2007; Bacheler et al., 2009b). However, our understanding of the movement patterns of red drum lags far behind our knowledge of mortality rates and habitat use, despite the fundamental implications of movement to the ecology and sustainable management of the species. For example, movement patterns have been used to determine the appropriate spatial scale of management (i.e., stock structure; Metcalfe, 2006) and to provide information on the ways juveniles move from nurseries to adult habitats (Beck et al., 2001; Gillanders et al., 2003).

The available literature on red drum movement is mixed. Studies conducted over relatively small spatial and temporal scales indicate that subadult red drum movement is limited (Collins et al., 2002; Dresser and Kneib, 2007). It also appears that adult red drum return to their natal estuary to spawn (Patterson et al., 2004). However, genetic differences exist only at very coarse scales (i.e.,

Gulf of Mexico and northwest Atlantic Ocean), not at finer spatial scales (Gold et al., 1993; Seyoum et al., 2000). This apparent discrepancy, whereby subadults show limited movements but genetic makeup is relatively homogeneous within each ocean, can potentially be explained in three ways. First, dispersal of larvae may occur over long distances along the coast, although evidence indicates that red drum spawn in inlets and estuaries (Johnson and Funicelli, 1991; Barrios, 2004; Luczkovich et al., 2008) where larvae are likely locally retained (Chen et al., 1997). Second, despite low movements rates (km/day) by subadults, adult movements may be high enough that genetic variability is homogenized at a basin-wide scale; this hypothesis remains untested because adult movement patterns have not been quantified. Third, in previous examinations of subadult red drum movement at relatively small temporal and spatial scales the full extent of subadult movements may have been missed.

We quantified the large-scale movements of subadult and adult red drum (using 25 years of conventional tagging data) and small-scale movement of subadult red drum (using three years of ultrasonic telemetry data. The specific objective of this work was to examine the effects of age, season, and region on movement patterns of North Carolina red drum. Potential differences in red drum movements by age, region, or season have implications for various aspects of the management (e.g., stock structure, spatial or temporal fishery closures, selectivity patterns) and ecology of the species (e.g., timing and spatial scale of gene flow and population connectivity). We used a variety of quantitative approaches to describe subadult and adult movement patterns, including ultrasonic telemetry, geographic mapping, and circular mapping. This study improves our understanding of the movement of red drum in estuarine and coastal waters of North Carolina and estuarine fish species more generally, and also provides some analytical techniques that are more widely applicable.

Materials and methods

Conventional tagging

Two sources of conventional tagging data were used. The first source was from a tagging study conducted by the North Carolina Division of Marine Fisheries (NCDMF) between 1983 and 2007 when red drum were captured opportunistically with pound nets, hook-and-lines, runaround gill nets, trammel nets, and by electrofishing. Volunteer recreational and commercial fishermen also participated in tagging red drum. The second data source was from a tagging study of subadult red drum during 2005–2007 conducted by North Carolina State University (NCSU) personnel within the lower Neuse River estuary (Bacheler, 2008). In both of these studies, only healthy fish were tagged and released.

Most subadult fish were tagged with internal anchor tags (Floy®, Seattle, WA; FM-84, FM-89SL, and FM-95W) and nylon dart tags (Floy® FT-1 and FT-2), whereas adults were primarily tagged with stainless steel dart tags containing a monofilament core (Floy® FH-69) or, more recently, containing a stainless steel core (Hallprint® SSD wire-through, Victor Harbor, Australia). All tags were labeled with a unique tag number and a "reward" message. All tag types were combined and treated equally in this study. The tag recovery location was either provided as latitude and longitude by fishermen or was estimated from the physical description provided by fishermen. For fishery-dependent tag recoveries, it was assumed that fishing effort was homogeneous over space, the implications of which are elaborated upon in the *Discussion* section.

We used a 6-mo age-length key developed by NCDMF to convert total length of fish at tagging to an estimated age based on a 1 January birthday. The age-length key was based on 17 years of North Carolina red drum ages that were estimated from otoliths, the annuli of which had been validated by Ross et al. (1995). A 6-mo age-length key (January–June and July–December) was used because of rapid summer growth rates that subadult red drum experience in North Carolina waters (Ross et al., 1995). The key provided very good separation of length-groups for fish younger than age 4. Sexually mature red drum were grouped into a single age-bin (age 4 and older [4+]; Ross et al., 1995). Thus, we used four age-groups (ages 1, 2, 3, and 4+) for all analyses. Lengths of fish were grouped into the four age bins as follows: for January–June, age 1: 0–253 mm, age 2: 254–558 mm, age 3: 559–761 mm, and age 4+: greater than 761 mm; for July–December, age 1: 0–507 mm, age 2: 508–710 mm, age 3: 711–812 mm, and age 4+: greater than 812 mm. In previous aging studies of adult red drum in North Carolina, maximum age was determined to be 62 years (Ross et al., 1995), indicating that age-4+ red drum in our study potentially ranged from age 4 to greater than 60.

Specific fishery regulations for red drum in North Carolina should not be a major source of bias in age-specific movements. The fishery regulation history for red drum is complex in North Carolina (see Bacheler et al. [2008a] for details), and currently only fish within a window limit (= a size limit with minimum and maximum length requirements, i.e., 457–686 mm TL, corresponding to ages 1–3) can be harvested legally. However, all ages of red drum are encountered in various (popular and targeted) catch-and-release recreational fisheries, and there are no major temporal or spatial restrictions on these fishing efforts.

To understand the generality of movement patterns of red drum in North Carolina waters, we first tested for differences in movement patterns among four regions (Fig. 1). These regions were the following: 1) eastern Pamlico Sound and the adjacent coastal waters (EPS; the outer banks from the Virginia state line to Cape Lookout), 2) western Pamlico Sound (WPS; waters near mainland areas of northern North Carolina), 3) Neuse

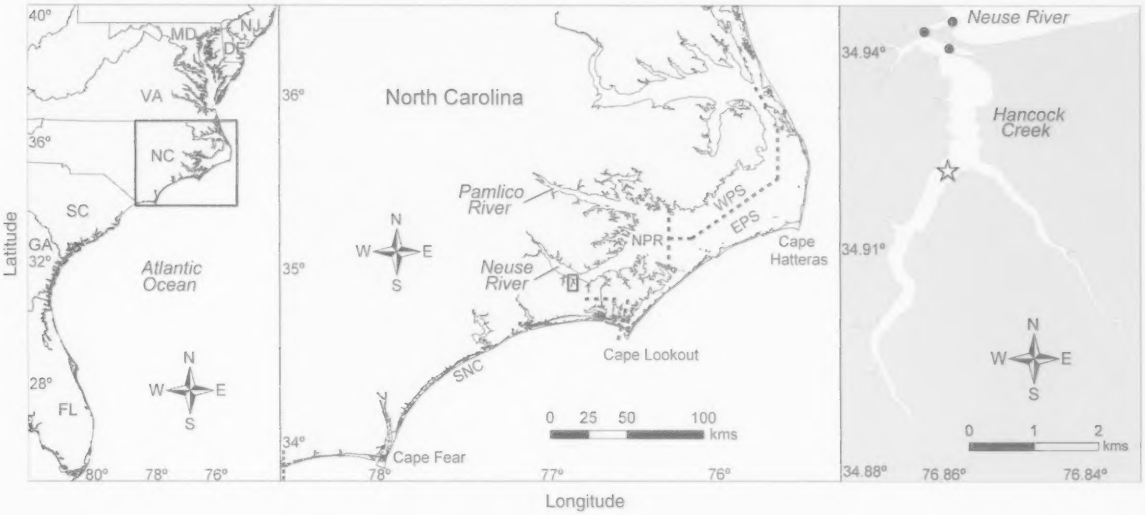

Figure 1

Map of study areas for red drum (*Sciaenops ocellatus*) within coastal and estuarine waters of North Carolina. Left map shows location of coastal North Carolina (in box) along the Atlantic coast of the United States. Middle map shows view of entire coastline of North Carolina, with the four regions used in the movement analyses demarcated by dashed lines. The four regions are the following: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and coastal and estuarine waters of southern North Carolina (SNC). The small box in the Neuse River highlights the location of Hancock Creek, which is enlarged in the right panel. Locations of submersible receivers in Hancock Creek are shown by the black dots, and the star shows where salinity measurements were taken.

and Pamlico rivers (NPR), and 4) waters of southern North Carolina (SNC; Cape Lookout southward, including estuaries and coastal waters). These regions were chosen from a preliminary examination of movement patterns of red drum and according to natural geographic divisions.

The latitude and longitude of tagging and recovery locations were used to calculate the distance (km) and angle moved (measured in whole-circle bearing degrees, with 0° representing true north). We calculated distance moved both as shortest distance moved in water, using ArcGIS 9.1 (ESRI, Redlands, CA) for distance and movement rate calculations, as well as straight-line distance (Batschelet, 1981) for circular mapping analyses. We also calculated the angle moved (in degrees) by each individual fish from the tagging and recovery coordinates (Batschelet, 1981).

Next, we tested for the effects of fish age, region, and season of conventional tagging on red drum movement patterns. We were unable to examine the simultaneous influence of these three factors on red drum movement patterns because of low sample sizes of recovered red drum in some age, region, and season combinations. Instead, we conducted two separate statistical analyses. In the first, we tested for differences in days at large, distance moved (km), and movement rate (km/d) among red drum age classes and regions of tagging, using analysis of variance (ANOVA). Each dependent variable was log-transformed to reduce skewness and to homogenize variability. Two-way factorial ANOVAs were used to test the main effects of age and region of tagging and their interaction at α=0.05. To visualize these age and region patterns, we first constructed maps of tagging and recovery locations for each age class of red drum, using ArcGIS 9.1. We next constructed two-variable vector plots in Oriana 2.0 (Kovach Computing Services, Anglesey, Wales). The length of the bars in these circular plots represents the straight-line distance moved by individual red drum, and the direction of the bar represents the angular bearing of the fish. Separate graphs were made for each age class and region combination. We were unable to use circular statistics on these data because of the presence of multiple modes (Zar, 1999) and geographic barriers that varied by region.

For the second statistical analysis we tested the influence of tagging season and age class on movement rates of red drum. Age was also included as a variable in this analysis because of its potential influence on seasonal movements. Only red drum recovered within 60 days of tagging were included in this analysis so that fish could be classified accurately into a seasonal period, and the midpoint of time at large for each fish was used to determine its season of recovery. Seasonal periods were classified as spring (March–May), summer (June–August), fall (September–November), and winter (December–February). Differences in log-transformed movement rates by season and age class were tested with a two-way factorial ANOVA.

To visualize this seasonal effect, stacked and stepped histograms of distance moved and directionality were

created within the circular plot. Age-1 and age-2 red drum were examined only in these plots because of low sample sizes of older age classes. The overall length of each wedge in the plot was the relative frequency of angular observations within 20° bins scaled to the largest number for each plot (because sample size was highly variable). Each wedge was further subdivided into the proportion of movements in various distance categories. These unique diagrams allowed for an examination of both direction and distance moved by season for red drum.

Anecdotal reports have indicated that some adult (age-4+) red drum migrate from coastal North Carolina waters northward each spring to Virginia and Maryland, and return southward in the fall. We examined the hypothesis of a seasonal migration of adult red drum with data from three sources: 1) the National Marine Fisheries Service (NMFS) trawl surveys; 2) Virginia Institute of Marine Sciences (VIMS) shark longline surveys; and 3) locations of tagged and recovered adult red drum in coastal waters from North Carolina northward. The NMFS trawl surveys were conducted in spring (March) and fall (September–November) in coastal waters from just south of Cape Hatteras northward (Despres-Patanjo et al., 1988); we used data from 1972 to 2004. The VIMS shark longline surveys have been conducted in the lower Chesapeake Bay and Virginia coastal waters from May or June through September or October (Conrath and Musick, 2007), and data from 1974 to 2004 were used. Data from these three sources were combined to provide a seasonally based map of adult red drum captures along the mid-Atlantic coast.

Ultrasonic telemetry

In order to quantify small-scale movements of subadult red drum, we also used ultrasonic telemetry data in a small lateral tributary of the Neuse River, Hancock Creek. Ultrasonic telemetry data were used in Hancock Creek instead of over a broader area (e.g., Pamlico Sound) because of the accessibility of the creek, ease of tracking, and its narrow mouth that could be monitored with submersible receivers (see below). Age-2 red drum were implanted with transmitters in Hancock Creek between 2005 and 2007; the surgical procedures are described in Bacheler (2008). Fish were surgically implanted with coded ultrasonic transmitters (VEMCO, Ltd., Halifax, Nova Scotia, Canada; V16 4H, 10 g in water; 10 mm wide; 65 mm long) and were released after swimming behavior returned to normal (approximately 10 minutes). Transmitter weight in water was always less than 1.25% of the fish's body weight, as recommended by Winter (1996); there was no evidence that the transmitter affected the behavior of red drum in the laboratory or field (Bacheler et al., 2009a). The transmitters operated on a frequency of 69 kHz and were programmed to be continuously active for a period of 641 days.

Transmitter-tagged red drum were manually tracked (relocated) monthly during daylight hours by using a VEMCO VR100 receiver and hydrophone. Upon relocation of a transmitter-tagged fish, the latitude and longitude coordinates were recorded, and water depth, temperature, salinity, and dissolved oxygen measurements were taken with a YSI® 85 (Yellow Springs Instruments, Inc., Yellow Springs, OH). Monthly movement rates were calculated as the shortest distance in water (km) between two successive relocation events. Upstream or downstream movements were determined for fish moving greater than 50 m in an upstream or downstream direction from its previous monthly location; otherwise, the fish was classified as stationary.

In order to quantify the seasonality and magnitude of emigrations from Hancock Creek, submersible VR2 VEMCO receivers were deployed at its mouth. Preliminary testing indicated that VR2 receivers could detect nearly 100% of the pulses from V16 tags at 400 m in our study system (Bacheler, 2008). Therefore, three submersible receivers were deployed at the mouth of Hancock Creek, each being a conservative distance of 600 m apart from one another. If a fish emigrated from the tributary, it was eliminated from the movement analyses.

We were also interested in potential correlations between movements and emigrations of red drum in Hancock Creek. Preliminary observations of transmitter-tagged red drum in Hancock Creek indicated that fish often moved in synchronized ways upstream or downstream during monthly periods. To test for seasonal effects, we related the frequency of fish moving upstream, moving downstream, and remaining stationary in Hancock Creek with the month of relocation, using an $R \times C$ test of independence. Given that the salinity regime in Hancock Creek was near the lower limit for red drum (i.e., 0–10 psu; Crocker et al., 1983; Forsberg and Neill, 1997), we also tested whether fluctuations in salinity were correlated with upstream and downstream movements, as well as with emigrations, of age-2 red drum. We correlated the proportion of tagged red drum moving upstream or downstream each month with the observed change in salinity near the midpoint (boat ramp) of Hancock Creek (see Fig. 1 for location) using Pearson's product-moment correlation analysis. Months with sample sizes of less than four tagged red drum were excluded from analysis.

Results

Conventional tagging

A total of 48,136 red drum (142–1473 mm total length) were tagged with conventional tags (i.e., internal anchor or dart) in this study, of which 6173 were recovered and reported by fishermen (Table 1). Overall, 58% of these recoveries were from fish tagged at age 1, 30% were from age 2, 2% were from age 3, and 9% were from age 4+ fish. A majority of recoveries occurred from fish originally tagged in the NPR (59%), but many fish were also recovered from releases in other regions as well

(Table 1). Some age and region combinations had small sample sizes, in particular age-3 and age-4+ red drum tagged and recovered in WPS.

Red drum were tagged and recovered broadly in nearshore areas throughout estuaries and the coast of North Carolina (Fig. 2). Age-1 and age-2 red drum were tagged in large numbers in all estuarine and coastal regions of the state, and recoveries occurred throughout North Carolina waters (Fig. 2, A and B). Tagging of age-3 red drum was mainly focused in NPR and EPS, and recoveries generally occurred in nearby areas (Fig. 2C). Tagging of age-4+ red drum was concentrated around Ocracoke Inlet (EPS), the lower Neuse and Pamlico rivers (NPR), and Cape Fear River (SNC); recoveries appeared to be concentrated in these same three areas (Fig. 2D).

Most recoveries occurred within the region in which tagging took place (Table 2). Highest regional fidelity was observed in SNC, where between 94% and 100% of tagged red drum were recovered in SNC. Lowest regional fidelity was observed in WPS, where from 0 to 3% of each age class were recovered in WPS; most fish tagged in WPS were recovered in EPS (50–100% of each age class; Table 2).

There were regional differences in movement metrics among the four age classes of red drum. Log-transformed mean days at large, mean distance moved, and mean movement rate of red drum were all significantly influenced by both region of tagging and age of the fish (two-way factorial ANOVA; all $P<0.001$). Specifically, mean days at large was positively related to age at tagging; age-1 red drum spent 100.8 ±2.8 d (mean ± standard error [SE]) at large, whereas age-4+ fish were at large much longer (693.8 ±37.9 d; Table 3). Mean distance moved was smallest for age-3 red drum (10.1 ±1.2 km) and farthest for age-4+ fish (30.2 ±2.0 km). Movement rates were much higher for age-1 red drum (1.1 ±0.1 km/d) compared to other age classes, which varied from 0.2 to 0.4 km/d (Table 3). In addition, there were significant interactions between region and age for all analyses (all interactions $P<0.01$).

Prevailing directions of movements were region- and age-dependent (Fig. 3). Generally, age-1 and age-2 red drum moved parallel to the coast (in estuarine and coastal waters), except for fish tagged in NPR, which tended to move primarily toward the coast. Rarely did subadult red drum move up rivers and estuaries toward low-salinity waters. Age-1 red drum tagged in EPS and WPS moved mainly southwest along the coast, whereas those tagged in SNC moved mainly northeast and southwest. Age-2 red drum generally showed more northward movements than age-1 red drum, especially in the northern regions of EPS and WPS (Fig. 3). Age-3 red drum displayed limited movements, but sample sizes for this age class were smaller than those for other age classes. Age-4+ red drum tagged in EPS moved farthest toward the north and south, but many fish moved shorter distances to the east and west. Movement distances for age-4+ red drum were minimal in all other regions, with the exception of primarily northeast (downriver) movements in NPR (Fig. 3).

Table 1

Number of conventionally tagged North Carolina red drum (*Sciaenops ocellatus*) that were recovered by fishermen and classified by age, region, and season of tagging, 1983–2007. No winter or spring data exist for age-1 red drum because these individuals were too small to be tagged in the winter and spring tagging program for this age group. Region codes are the following: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and southern North Carolina (SNC).

Season of tagging by age	Region				
	EPS	WPS	NPR	SNC	Total
Age 1					
Summer	179	58	871	104	1212
Fall	340	200	1,550	220	2310
Age 2					
Winter	184	48	356	79	667
Spring	171	13	523	47	754
Summer	98	6	102	29	235
Fall	119	20	59	99	297
Age 3					
Winter	12	0	24	2	38
Spring	17	2	4	9	32
Summer	13	0	0	16	29
Fall	40	1	2	7	50
Age 4+					
Winter	1	0	1	4	6
Spring	71	0	0	1	72
Summer	27	0	86	30	143
Fall	242	2	73	11	328
Total	1514	350	3651	658	6173

Red drum of all ages had highest movement rates during fall. Movement rate within 60 days of tagging was influenced by season ($P<0.01$) and age ($P=0.04$); the interaction between season and age was also significant ($P<0.01$). Age-1 red drum showed the highest fall movement rates, and age-4+ displayed the highest movement rate of any age class in spring and summer. Age-3 red drum had the lowest movement rate of any age class in spring and fall.

Four features were apparent for the detailed seasonal examination of directions and distances moved for age-1 and age-2 red drum (Fig. 4). First, higher proportions of long-distance movements occurred during fall months; in fact, a majority of movements during fall months consisted of distances greater than 20.1 km. Second, regional differences were observed in both distances and directions moved, especially during fall months. For instance, most movements of age-1 red drum tagged in eastern Pamlico Sound consisted of long-distance movements (>20.1 km) to the southwest,

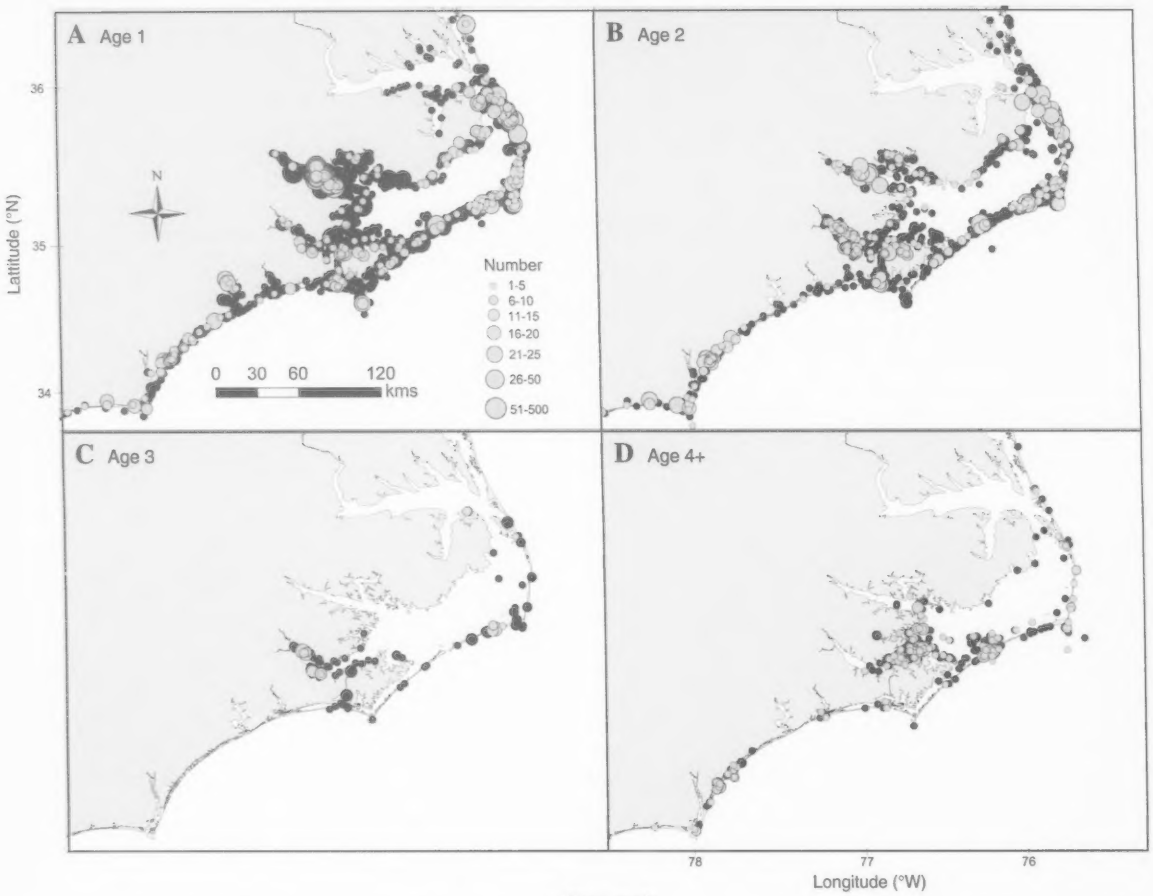

Figure 2

Tagging (gray circles) and recovery locations (black circles) for (**A**) age-1, (**B**) age-2, (**C**) age-3, and (**D**) age-4+ red drum (*Sciaenops ocellatus*) conventionally tagged by North Carolina Division of Marine Fisheries and North Carolina State University, 1983–2007. Only recoveries occurring within North Carolina waters are shown.

whereas age-1 fish in other regions moved primarily south (WPS), east (NPR), or northeast and southwest (SNC). Third, during winter, spring, and summer months, movements of subadult red drum tended to consist of short-distance movements of highly variable directionality. Fourth, when comparing age-1 and age-2 red drum movements in fall months, we found that the direction and distances of movements were mostly similar for EPS and WPS. However, age-specific differences were observed for NPR, which had more long-distance, coastward movements of age-2 than age-1 red drum, and for SNC, which had more westward movement of age-2 fish and highly variable movement of age-1 red drum.

Thirty-six red drum were recovered in states other than North Carolina (0.6% of all recoveries). Most out-of-state recoveries were from fish tagged at age 2 (56%), but some had fish been tagged at age 1 (22%) or age 4+ (22%). Most out-of-state recoveries came from Virginia (78%), but recoveries also occurred in South Carolina (11%), Maryland (5%), Georgia (3%), and Delaware (3%). Most out-of-state recoveries came from fish tagged in EPS (82%), but some also came from NPR (6%), SNC (6%), and WPS (6%).

Catches of adult red drum in coastal waters of North Carolina, Virginia, and Maryland from the NMFS and VIMS fishery-independent surveys showed a seasonal geographic pattern (Fig. 5). In March, adult red drum were located exclusively on the continental shelf of North Carolina. Adult red drum were encountered in lower Chesapeake Bay and coastal Virginia and Maryland in spring and summer months, and catches during late fall were centered back in North Carolina.

Ultrasonic telemetry

In total, 105 age-2 red drum were implanted with transmitters in Hancock Creek from March 2005 to December

Table 2

Region of tagging and number of tagged red drum (*Sciaenops ocellatus*) recovered within North Carolina estuarine and coastal waters, 1983–2007. Region codes are the following: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and southern North Carolina (SNC).

Age of fish and region of tagging	Region of tag recovery					Total
	EPS	WPS	NPR	SNC	Out-of-state	
Age 1						
EPS	429	4	7	73	6	519
WPS	236	5	11	5	1	258
NPR	234	1	2119	66	1	2421
SNC	8	1	12	303	0	324
Age 2						
EPS	524	12	10	9	17	572
WPS	80	3	2	1	1	87
NPR	158	4	813	64	1	1040
SNC	6	3	1	243	1	254
Age 3						
EPS	78	0	2	2	0	82
WPS	3	0	0	0	0	3
NPR	1	0	27	2	0	30
SNC	0	0	0	34	0	34
Age 4+						
EPS	225	11	94	4	7	341
WPS	1	0	1	0	0	2
NPR	29	3	127	1	0	160
SNC	1	0	1	43	1	46
Total	2013	47	3227	850	36	6173

Table 3

Summary of movement information for four age groups of red drum (*Sciaenops ocellatus*) in estuarine and coastal waters of North Carolina based on their age at tagging, 1983–2007. Standard errors (SE) are show in parentheses. Age-1 and age-2 red drum were analyzed within each of four tagging regions: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and southern North Carolina (SNC). Age-3 and age-4+ red drum movement information was summarized across all regions because of low sample sizes in some regions. Shortest distance in water was used for all distance and movement rate calculations.

Age of fish and region of tagging	Total recoveries	Mean (SE) days at large	Maximum days at large	Mean (SE) distance moved (km)	Maximum distance moved (km)	Mean (SE) movement rate (km/d)	Proportion moving <10km
Age 1							
EPS	519	134.2 (7.4)	1079	49.0 (2.3)	353.5	2.0 (0.2)	0.32
WPS	258	186.7 (15.3)	1532	44.7 (2.5)	314.5	2.4 (0.5)	0.25
NPR	2421	77.9 (3.0)	1882	21.0 (0.6)	202.6	1.0 (0.1)	0.66
SNC	324	147.3 (9.3)	1125	27.8 (2.4)	306.7	0.5 (0.1)	0.49
Overall	3522	100.8 (2.8)	1882	24.9 (0.6)	353.5	1.1 (0.1)	0.56
Age 2							
EPS	572	164.8 (7.6)	2056	28.7 (1.9)	622.5	0.7 (0.1)	0.45
WPS	87	179.4 (13.7)	621	43.3 (3.7)	166.7	0.8 (0.1)	0.21
NPR	1040	150.5 (4.0)	816	20.4 (1.0)	220.7	0.2 (0.1)	0.57
SNC	254	151.2 (12.8)	1043	11.9 (2.2)	186.9	0.3 (0.1)	0.74
Overall	1953	155.6 (3.5)	2056	22.4 (0.8)	622.5	0.4 (0.1)	0.54
Age 3	149	176.7 (35.6)	4752	10.1 (1.2)	80.0	0.2 (0.1)	0.68
Age 4+	549	693.8 (37.9)	5955	30.2 (2.0)	305.8	0.3 (0.1)	0.47

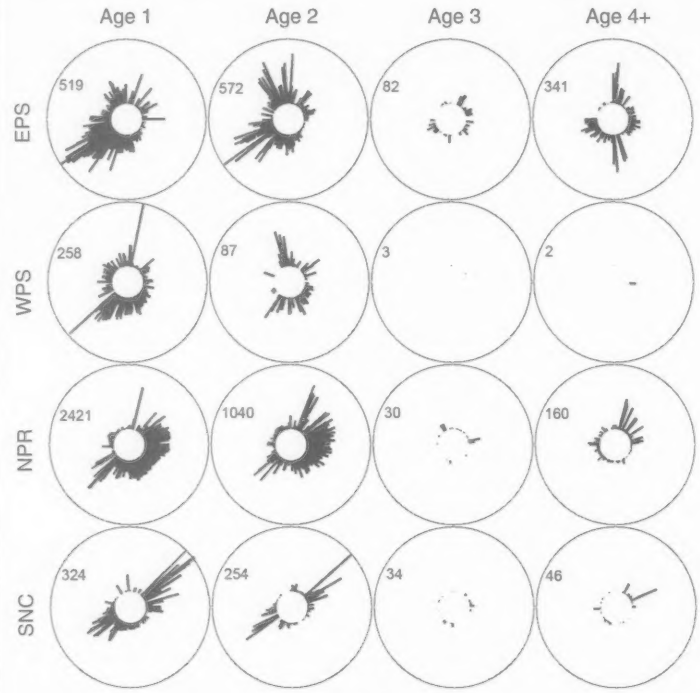

Figure 3

Rose diagrams showing the direction and distances moved for conventionally tagged red drum (*Sciaenops ocellatus*) in the coastal and estuarine waters of North Carolina, 1983–2007. Age classes are shown in columns and region of tagging in rows. The four regions are the following: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and southern North Carolina (SNC). Northward movements are straight up, southward movements are straight down, and outer circle is scaled to 300 km. Sample size is provided on each diagram.

2007. Most (77%) ultimately emigrated from the system, but some were harvested by fishermen (15%) and others remained alive at the end of the study (7%). One fish (1%) died from the surgical tagging procedure.

Emigration rates from Hancock Creek were bimodal and seasonal (χ^2=41.6; P<0.001; Fig. 6A), with most fish emigrating in spring (April–June) or fall months (September–November). No fish emigrated during winter (December–February). Movement rates of tagged red drum within Hancock Creek (i.e., excluding movements of emigrating fish) were also seasonal (ANOVA: P=0.01); highest movements occurred in May and lowest in January and February. Lastly, directionality of movements in Hancock Creek was dependent upon month of relocation (R×C test of independence: χ^2=53.4, P<0.001), but no obvious seasonal trend was observed (Fig. 6B).

Upstream and downstream movements of transmitter-tagged red drum were also significantly correlated with fluctuations in salinity (Fig. 7). The proportion of red drum moving upstream was correlated with a positive monthly change in salinity (r=0.57; F=9.19; P=0.007; Fig. 7A), and, similarly, downstream movements were correlated with a negative monthly change in salinity (r=–0.68; F=16.28; P<0.001; Fig. 7B). Changes in salinity did not influence emigration rate, however (r=–0.20; F=0.19; P=0.67; Fig. 7C).

Discussion

Our analyses on the movement patterns of red drum are innovative and of broad interest for two reasons. First, we provided a thorough treatment of the multiscale movement patterns of subadult and adult red drum by using multiple techniques and sources of data over many years, resulting in a comprehensive examination of movement of an estuarine fish species. Second, we

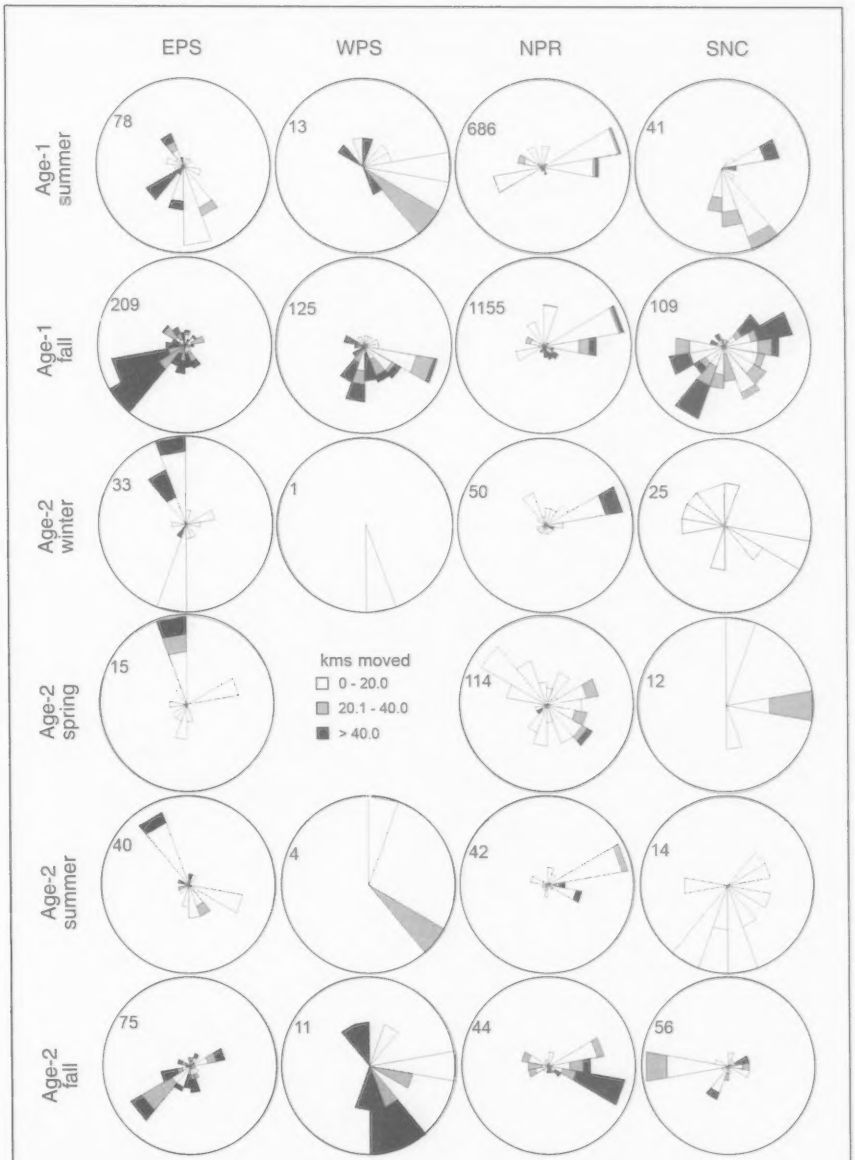

Figure 4

Frequency distributions of angular directions moved for subadult red drum (*Sciaenops ocellatus*) recovered within 60 days of tagging in coastal and estuarine waters of North Carolina, 1983–2007. Season and age class shown as rows and region tagged as columns. The four regions are the following: eastern Pamlico Sound (EPS), western Pamlico Sound (WPS), Neuse and Pamlico rivers (NPR), and southern North Carolina (SNC). The overall length of each wedge shows the relative frequency of angular observations within 20° bins scaled to the largest number for each plot. Each wedge is further subdivided into the proportion of movements in a particular direction composed of distances less than or equal to 20 km (white), 20.1 to 40.0 (gray), or greater than 40 km (black). Northward movements are straight up, and southward movements are straight down. Sample size is given for each diagram.

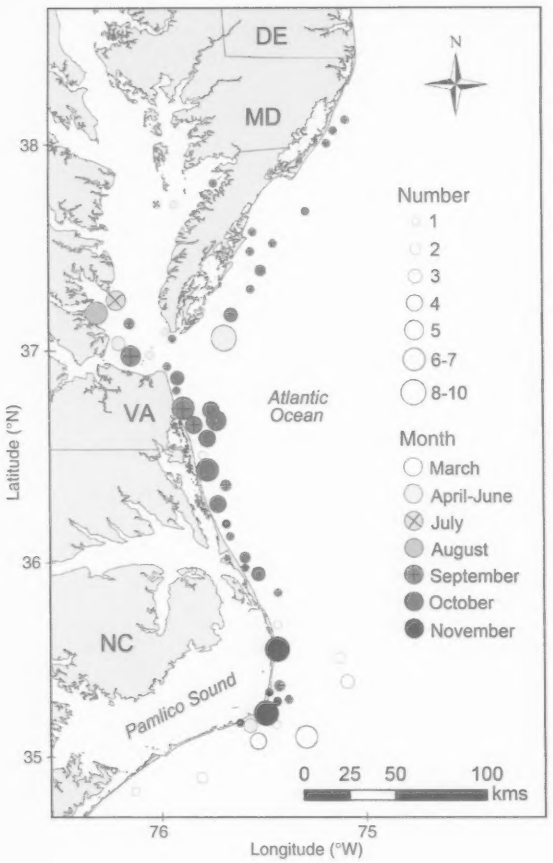

Figure 5

Seasonality and numbers of captures of adult (age-4+) red drum (*Sciaenops ocellatus*) caught in coastal North Carolina, Virginia, and Maryland. Data are from the National Marine Fisheries Service trawl surveys, Virginia Institute of Marine Sciences shark longline surveys, or from fish tagged or recovered in the North Carolina Division of Marine Fisheries tagging project (1983–2007). The National Marine Fisheries Service survey took place in U.S. east coast continental shelf waters from Gulf of Maine to just south of Cape Hatteras in spring (March) and fall (September–November) each year in 1972–2004, and the Virginia Institute of Marine Sciences survey Virginia Institute of Marine Sciences survey was conducted in Chesapeake Bay and coastal Virginia waters in May or June through September or October, 1974–2004.

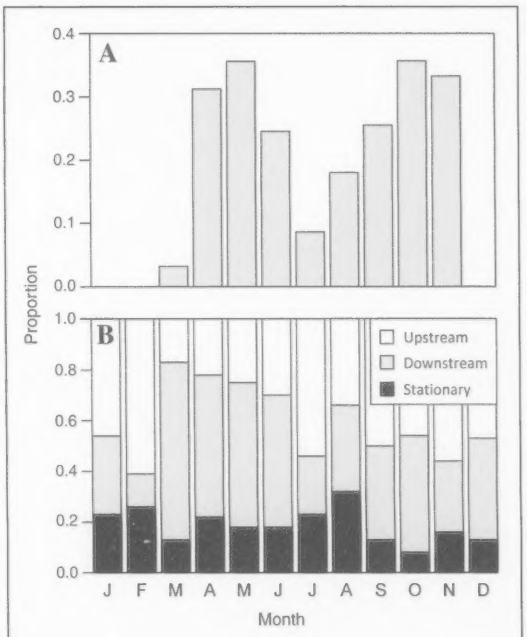

Figure 6

Seasonal ultrasonic telemetry information for age-2 red drum (*Sciaenops ocellatus*) in Hancock Creek, 2005–07. (**A**) Proportion of transmitter-tracked red drum emigrating each month from Hancock Creek, combined across years. (**B**) Proportion of tracked red drum moving upstream, downstream, or remaining stationary within Hancock Creek, combined across years.

developed a series of intuitive figures (e.g., circular mapping) to summarize the ways in which the movement patterns of an estuarine fish species were influenced by a variety of factors. Our results have implications for stock structure, gene flow, and ultimately, the connectivity of estuarine fish populations.

Movement patterns of red drum were distinctly age-dependent. Rates of movement generally declined with age, although the estimates for adult fish may be low if these adult fish were encountered mostly in estuarine waters after returning to spawn. From a physiological perspective, red drum are expected to show preferences for higher salinity with age (Neill et al., 2004), which may at least partially explain the observed age-dependent movement patterns towards the coast. Red drum are also known to experience major ontogenetic shifts in diet and habitat use (Bacheler et al., 2009b), but it is unknown how these ecological shifts translate to age-dependent and seasonal movement patterns. Although movement rates of many fish species have been shown to be age-dependent (e.g., Skalski and Gilliam, 2000), previous work on the movements of red drum focused on only one age class (Dresser and Kneib, 2007) or found no differences among age groups (Osburn et al., 1982). The observation that red drum movement patterns are age-specific is important for explaining age-specific selectivity patterns of the fishery (Bacheler et al., 2008a). In addition, the timing of movement for each age class can also be used to create temporal closures as a fishery management tool to protect red drum during particularly vulnerable periods when movement rates are high (e.g., to protect them from passive gear like gill nets).

The use of multiple tag types and age-dependent selectivity patterns in our study may have biased our analyses of movement patterns of fish by age. Most subadult red drum were tagged with internal anchor tags, which have been shown to have higher retention rates than dart tags that were primarily applied to adult red drum in our study (Bacheler et al., 2008a). Selectivity appears to be dome shaped and centered upon subadult red drum within the window limit for red drum catch (Bacheler et al., 2008a). Unequal retention rates of tags and age-dependent selectivity patterns likely did not bias movement rates or distances moved but may have biased our analysis of days at large. It is likely that adult red drum would have shown even greater differences in days at large compared to subadult fish if a tag with greater retention had been used for adults and there would have been increased selectivity on adult red drum during fewer days at large.

Determining whether estuarine or coastal species exhibit seasonally dependent movements is an important step in developing a broader perspective on the ecology of a particular species. The limited temporal scope and modest sample size of previous estuarine tagging studies have made it difficult to quantify seasonal variability in movement patterns of estuarine fish species like red drum. We documented a high rate of (primarily southward) movement by age-1 red drum during fall months, especially in northern regions of North Carolina (EPS and WPS); North Carolina happens to be the most significant northern overwintering grounds for subadult red drum on the Atlantic coast (Ross et al., 1995). Atlantic silversides (*Menidia menidia*) are known to migrate offshore in the northern but not the southern part of their range in the Atlantic (Conover and Murawski, 1982), presumably to avoid overwintering mortality due to acute cold stress in northerly latitudes (Munch et al., 2003). Adult bluefish (*Pomatomus saltatrix*) on the Atlantic coast appear to consist of three groups that exhibit different migratory behaviors; the group inhabiting the most northerly waters (i.e., New England) in summer months tends to exhibit the farthest southerly migration during fall (Shepherd et al., 2006). Likewise, southerly movements of age-1 red drum during fall months may be an avoidance response to acute cold stress (e.g., Gunter, 1941) that may be particularly hazardous in the northern part of the state.

Despite the low sample size of conventionally tagged and recovered red drum in some age and region combinations, regional variability was apparent. Regional variability in the movement of tagged estuarine fish is likely a result of the physiology of the species, geographic barriers, and the specific fisheries operating in each region. In addition to the seasonal movements described above for age-1 red drum in northerly regions of the state, there appeared to be a coastward (easterly) migration for both age-1 and age-2 fish tagged in oligohaline waters, whereas fish tagged in polyhaline waters primarily moved along the coast. Regionally variable movements may be due as much to the physiological requirements of red drum as to the geography of the North Carolina coast, which constrains the movements of red drum to specific directions (e.g., east–west in NPR, northeast–southwest in SNC).

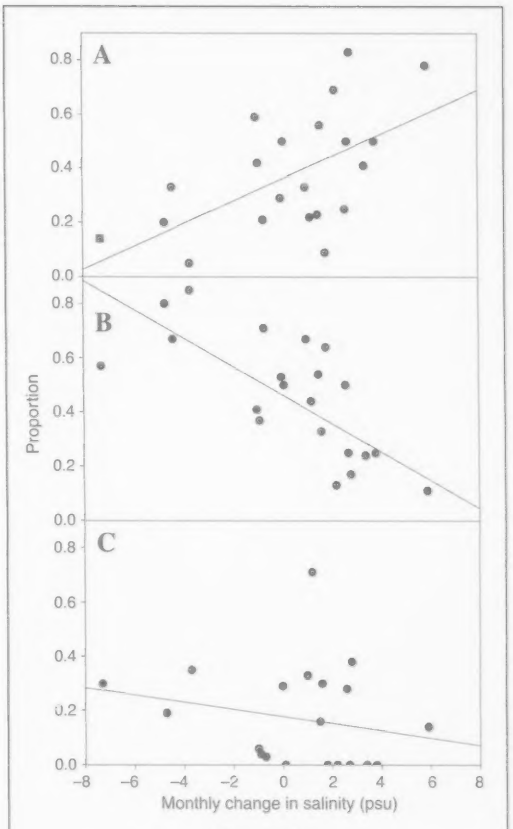

Figure 7

Proportion of transmitter-tracked red drum (*Sciaenops ocellatus*) in Hancock Creek moving upstream (**A**), moving downstream (**B**), or emigrating (**C**) in relation to the change in salinity between two consecutive monthly relocation periods, 2005–2007. Salinity sampling took place midriver near the boat ramp, and monthly periods were included only if at least four tracked red drum were relocated in that period. Trend lines show a linear least-squares fit.

Because tag recoveries come from the fishery, conventional tagging analyses of movement can be biased by spatially heterogeneous fishing effort. The distribution of recoveries may therefore reflect the spatial distribution of fishing more than the true extent of fish movement. Bolle et al. (2005) used electronic transmitters to show that conventional tagging provided a reliable interpretation of the movement patterns of European plaice (*Pleuronectes platessa*) in most areas of the North Sea; the only areas that appeared to be undersampled

by conventional tagging were places where residence time was short, fishing effort was low, and catchability was reduced. We could not compare conventional tagging data with ultrasonic telemetry data in our study because the spatial distribution data from the conventional tags and the ultrasonic telemetry tags did not overlap. However, we believe movement data from conventional tagging were generally robust except for fish tagged in the NPR. A large number of fish were conventionally tagged in conjunction with commercial pound-net operations in the Pamlico River, and many were recovered within a few days in the same or nearby pound nets. Such intense localized fishing pressure adjacent to major tagging operations likely biased NPR movement data, resulting in shorter mean distances moved and days at large than mean distances and days at large from other regions where fish were not tagged from pound nets. In light of the unusual pound-net tagging in the Pamlico River, movement data from NPR should be viewed cautiously. Most recoveries of fish tagged in WPS occurred in EPS, but this result is not surprising given that the primary WPS tagging location was near the dividing line between the two regions. Based on available information, WPS has a similar level of fishing activity as that of other areas of North Carolina.

Our analyses could have been improved if fishing-effort data across coastal North Carolina had been available. Because heterogeneous fishing effort may influence movement results, tag recoveries have been standardized by regionally variable fishing effort in recent movement analyses (e.g., Wang et al., 2007). Building upon the pioneering work of Hilborn (1990), McGarvey and Feenstra (2002) went further and developed a movement model that uses fishing effort or mortality data across space in a maximum likelihood framework to estimate the probability parameters of movement. Accurate fishing-effort data could be useful for future red drum tagging analyses and may improve both movement and mortality modeling.

The addition of an ultrasonic telemetry method in this study to examine small-scale movement patterns of subadult red drum complements large-scale analyses that use conventional tagging. When they could be compared, the two different methods provided similar movement patterns. For instance, the high emigration rate of age-2 transmitter-tracked red drum from Hancock Creek in fall months corresponded with the season when conventionally tagged age-2 fish moved at the highest rate. The direction of movement during fall months was also consistent for the two methods. From data from additional submersible receivers deployed both upstream and downstream of Hancock Creek (see Bacheler et al. [2009a] for description), we determined that most transmitter-tracked red drum (87%) emigrating during fall months moved downstream in the direction of the coast (senior author, unpubl. data). Likewise, conventionally tagged fish in NPR also moved downstream toward the coast during fall months.

Ultrasonic telemetry could also be used to relate smaller-scale movement patterns to environmental variability. Because the salinity in Hancock Creek (0–10 psu) is near the lower tolerance limit for red drum (Crocker et al., 1983; Forsberg et al., 1997), upstream and downstream movements of transmitter-tracked red drum may have been a physiological response to fluctuating salinities. In laboratory experiments, estuarine organisms have been shown to respond to changes in salinity with increased swimming speed and respiration (von Oertzen, 1984). Alternatively, transmitter-tracked red drum may have been following the movements of prey species (Bacheler et al., 2009b) that had their own physiological constraints. Regardless, transmitter-tracked red drum appeared to remain in salinities around 4–5 psu (Bacheler et al., 2009b), following this gradient up and down the creek with fluctuations in salinity. Our results contrast with those of Dresser and Kneib (2007), who showed that subadult red drum movement patterns in a coastal Georgia saltmarsh were primarily influenced by tide and time of day. The lack of lunar tides in Hancock Creek, in addition to much lower salinities, may explain this discrepancy.

We developed a conceptual diagram to highlight the ways in which our conventional tagging and ultrasonic telemetry data helped elucidate several critical aspects of red drum life history and ontogeny (Fig. 8). Spawning occurs in late summer (Barrios, 2004; Luczkovich et al., 2008), and fertilized eggs are advected upstream where they eventually hatch into pelagic larvae and settle to benthic nursery habitats during fall (Bacheler et al., 2008b). Age-0 to age-3 red drum are found in upper estuarine environments, but we have shown that each fall a portion of both age-1 and age-2 cohorts move to high-salinity coastal waters (Fig. 8). It appears that some red drum remain in upper estuary habitats until age 3, the age at which the last remaining red drum move to coastal environments. Subadult red drum in coastal environments join the adult population after maturity at age 3 or 4 (Ross et al., 1995). We have also shown that adults overwinter on the continental shelf and some move westward into North Carolina estuaries, whereas others move northward to the lower Chesapeake Bay or coastal Virginia and Maryland during spring, and back east or south during fall months. The large proportion of conventionally tagged adults recovered near their tagging location in summer months indicates a return to specific spawning areas each year. We could not eliminate the possibility that some adult red drum remain in continental shelf waters year-round and spawn on the shelf or in passes or inlets, as has been observed in another study (Murphy and Taylor, 1990). Therefore, the arrows in our conceptual diagram highlighting the seasonal movements of adult red drum into the estuary were dotted to acknowledge this uncertainty. Taken together, these movement results have direct implications for the use of temporal and spatial management tools and also for the scale at which management and assessment should take place.

Assessment of North Carolina and Virginia red drum together as one stock is justified by tagging data. Subadult red drum tagged with conventional tags in North

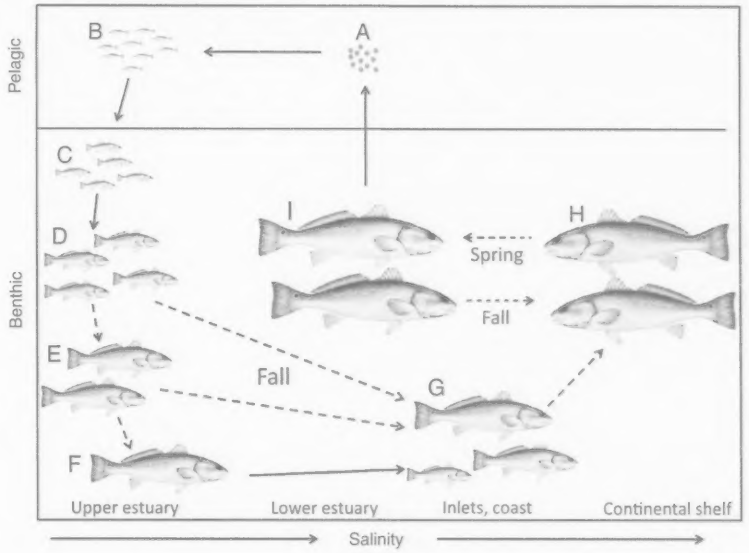

Figure 8

Conceptual diagram of life history and movement patterns of red drum (*Sciaenops ocellatus*) in North Carolina. (**A**) Eggs are spawned in August–September; (**B**) larvae are pelagic in August–November; (**C**) age-0 juvenile red drum settle to benthic habitats in upper estuaries in September–November; (**D** and **E**) age-1 and age-2 red drum either remain in upper estuaries or migrate downstream to coastal habitats in fall; (**F**) age-3 red drum migrate towards the coast throughout the year; (**G**), multiple age classes of subadult red drum inhabit coastal habitats, eventually joining adults after maturity at approximately age 3 or 4 years; (**H**) nonspawning adults on continental shelf (age-4+) overwinter; (**I**) adults spawn during summer months. Dotted lines indicate that a particular pathway is not necessarily followed by all members of a cohort in a particular year.

Carolina appear to be much more likely to move northward to Virginia than to any other state, even though interstate movements were low. Likewise, subadult red drum tagged in Virginia have consistently been recovered in North Carolina waters (J. Lucy, personal commun.[1]). Few subadult red drum were captured in states southward in our study, and, similarly, tagged red drum in South Carolina are rarely recovered in North Carolina (C. Wenner, personal commun.[2]). The interstate movement patterns of adult red drum appear to mirror those of subadults, showing some northward seasonal migration to Virginia each year, but very limited exchange with states south of North Carolina. Our tagging results indicate that the state line between North Carolina and South Carolina corresponds approximately to an ecological division for red drum and, thus, is an appropriate division for management and assessment of the stock.

We used a 25-year tagging data set in combination with three years of ultrasonic telemetry data and coastal fishery-independent survey data to provide a comprehensive examination of subadult and adult red drum movement. Red drum movement patterns in North Carolina were dependent upon the age, region, and season of tagging. Longitudinal movements of age-2 red drum within a tributary to the Neuse River were related to salinity fluctuations, but emigrations from the tributary were dependent on season and not salinity. These results advance our understanding of the seasonality, regional variability, age dependency, and spatial scale of movements of fish in complex estuarine and coastal environments.

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[1] Lucy, Jon. 2009. Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

[2] Wenner, Charles. 2009. South Carolina Department of Natural Resources, Charleston, SC 29412.

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Reproductive biology of blue marlin (*Makaira nigricans*) in the western Pacific Ocean

Chi-Lu Sun (contact author)
Yi-Jay Chang
Chien-Chung Tszeng
Su-Zan Yeh
Nan-Jay Su

Email address for contact author: chilu@ntu.edu.tw

Institute of Oceanography
National Taiwan University
1 Sec 4 Roosevelt Rd
Taipei, Taiwan 10617

Abstract—The reproductive biology of blue marlin (*Makaira nigricans*) was assessed from 1001 fish (ranging from 121 to 275 cm in eye-to-fork length; EFL) caught by Taiwanese offshore longliners in the western Pacific Ocean from September 2000 to December 2001 and from 843 gonad samples from these fish, The overall sex ratio of the catch was approximately 1:1 during the sampling period, but blue marlin are sexually dimorphic; females are larger than males. Reproductive activity (assessed by histology), a gonadosomatic index, and the distribution of oocyte diameters, indicated that spawning occurred predominantly from May to September. The estimated sizes-at-maturity (EFL_{50}) were 179.76 ±1.01 cm (mean ±standard error) for females and 130 ±1 cm EFL for males. Blue marlin are multiple spawners and oocytes develop asynchronously. The proportion of mature females with ovaries containing postovulatory follicles (0.41) and hydrated oocytes (0.34) indicated that the blue marlin spawned once every 2–3 days on average. Batch fecundity (BF) for 26 females with the most advanced oocytes (≥1000 μm), but without postovulatory follicles, ranged from 2.11 to 13.50 million eggs (6.94 ±0.54 million eggs). The relationships between batch fecundity (BF, in millions of eggs) and EFL and round weight (RW, kg) were $BF = 3.29 \times 10^{-12}\ EFL^{5.31}$ (r^2=0.70) and $BF = 1.59 \times 10^{-3}\ RW^{1.73}$ (r^2=0.67), respectively. The parameters estimated in this study are key information for stock assessments of blue marlin in the western Pacific Ocean and will contribute to the conservation and sustainable yield of this species.

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Blue marlin (*Makaira nigricans*) are widely distributed throughout the tropical and subtropical waters of the Pacific and Indian oceans (Nakamura, 1985). In the Pacific, blue marlin are harvested mainly by longline fisheries targeting tunas. Genetic studies (Buonaccorsi et al., 1999) and fishery data (Kleiber et al., 2003) indicate that there is a single stock of blue marlin in the Pacific Ocean. Annual landings of blue marlin in the western and central Pacific over the past decade have been stable at about 13,000 metric tons. However, stock assessments of Pacific blue marlin are uncertain, with results ranging from the stock being close to fully exploited (Kleiber et al., 2003), overfished (Yuen and Miyake, 1980), or in a healthy state (Hinton, 2001).

Quantifying the reproductive potential of blue marlin is important for understanding the population dynamics of this species and for stock assessment purposes. For example, estimates of the size- and age-at-sexual-maturity are necessary inputs for age- and size-structured stock assessments models (Quinn and Deriso, 1999). Despite the size of the fisheries for the stock of blue marlin in the Pacific Ocean, there have been few published studies on the reproductive biology of this stock. In the eastern Pacific, Kume and Joseph (1969) estimated the size-at-maturity of blue marlin from data from the Japanese longline fishery, and Hopper (1990) described spawning activity around the Hawaiian Islands. In the western Pacific, Nakamura (1944) reported that blue marlin spawned off Taiwan. However, none of these studies provided detailed information on ovarian development, even though knowledge of gonad development in individual fish is needed to establish the spawning season, the size- and age-at-maturity, and the spawning pattern. The reproductive biology of blue marlin has been studied more extensively in the Atlantic Ocean than in the Pacific. For example, La Monte (1958) first described the gonad of blue marlin in the Atlantic Ocean, Erdman (1968) observed the reproductive cycle off Puerto Rico, Cyr (1987) defined the gonad development and spawning cycle in the northwestern Atlantic Ocean, and Arocha and Marcano (2008) estimated the size-at-maturity of this species in the western central Atlantic. The objectives of this study were to evaluate the reproductive biology of blue marlin in the western Pacific Ocean. We determine reproductive activity and describe ovarian development using histological techniques. Key parameters required for stock assessments including sex ratio, reproductive season, and size-at-maturity are also estimated.

Materials and methods

Collections from fish markets and measurements of fish

Samples from 1001 blue marlin were collected randomly from September 2000 to December 2001 at the Tungkang fish market in southwest Taiwan. All samples were caught by offshore longliners operating between 16–23°N latitude and 115–135°E longitude. Sex (determined from the macroscopic characteristics of the gonads, and from histological sections for small individuals), eye-to-fork length (EFL; the posterior margin of eye's bony orbit to the distal end of the central ray of the caudal fin, cm), and round weight (RW, kg) were recorded for each fish. A tissue sample was randomly collected from the anterior (females) and middle (males) of either the right or left lobe of the gonad and immediately fixed in 10% buffered formalin for later oocyte measurement and histological analysis. Three ovary pairs were collected in July 2004 to evaluate the synchronicity of egg development within, and between, ovary pairs. The left and right lobes of these ovaries were each divided into anterior, central, and posterior portions, and each portion further divided into outer, middle, and central layers. A total of 54 0.05-g subsamples (3 ovaries×2 lobes×3 portions×3 layers) were collected randomly and the number of whole oocytes and the mean oocyte diameter (MOD) for each subsample was estimated. More specifically, the MOD was estimated as the average of the diameters of the most advanced group of oocytes calculated with the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD), after calibration against an optical micrometer. However, histological sectioning deforms the oocyte from its sphere-like shape, and three types of measurements were therefore used to obtain reliable results (Arocha, 2002). Early developed oocytes were measured at the major axis crossing the nucleus; maturing oocytes were measured across the nucleus from well-formed spheres; and the diameters of fully mature oocytes were calculated from the circumference of the oocyte divided by π.

The condition factor, C, was determined for each fish by using the relationship

$$C = (100{,}000 \times RW) / EFL^b, \quad (1)$$

where b = the slope of the length-weight relationship (King, 1995).

The value for b was estimated by using linear regression after the data were log-transformed. The parameter b was not significantly different from 3 for females (t=0.53, df=172, P >0.05) and males (t=−0.99, df=210, P >0.05). The condition factor was related to the gonadosomatic index (GSI), which was determined as follows (Uosaki and Bayliff, 1999):

$$GSI = (GW / EFL^3) \times 10^4, \quad (2)$$

where GW = gonad weight (g).

Sex ratio in each month or size class was calculated as the ratio of the number of females to the total numbers of females and males. Chi-square tests were used to test for significant differences in sex ratio among months and sizes. Sex ratios were also regressed on length by using logistic regression (DeMartini et al., 2000).

Reproductive activity

Microscopic characteristics of histological sections and the most advanced group of oocytes were used to assign ovaries to stage (Hunter and Macewicz, 1985; West, 1990; Arocha, 2002; Arocha and Bárrios, 2009). For males, the classification of testicular development was based on the degree of spermatogenesis, the development of the vas deferens, and the composition of germ cells (Grier, 1981; Ratty et al., 1990; deSylva and Breder, 1997). Each 150-mm^3 preserved tissue sample was embedded in paraffin, sectioned at 7 μm, and stained with Mayer's haematoxylin and eosin. The dynamics of the ovarian maturation process were evaluated by examining the modes in size-frequency distributions of whole oocytes (after Hunter and Macewicz, 1985, Arocha, 2002; Arocha and Bárrios, 2009). The 95% confidence intervals for the proportion of oocytes of each diameter by ovarian maturity stage were obtained by a bootstrap procedure in which each pseudo data set was constructed by selecting whole oocytes at random and with replacement. Changes over time in the mean diameter of the most advanced group of oocytes, the GSI values (for individuals larger than ≥180 cm EFL to enhance temporal variation), and the composition of ovarian development stages were evaluated to determine the spawning season.

Size-at-maturity

The proportion of mature fish of all assessed fish classes, defined as a maturity ogive, was developed from samples caught during the spawning season (Murua et al., 2003). Females were defined as sexually mature if they had early yolked, advanced yolked, migratory nucleus, or hydrated oocytes (Hunter and Macewicz, 2003; Arocha and Bárrios, 2009), whereas males were defined to be sexually mature if they had secondary spermatocytes, spermatids, or spermatozoa. The presence of postovulatory follicles (POF) in ovaries and spermatozoa in the vas deferens were respectively taken as evidence of recent spawning of females and males. Atresia of yolked oocytes was also noted for mature but reproductively inactive females. The probability that the i^{th} fish was mature (P_i) was modeled with a logistic curve:

$$P_i = 1 / \left(1 + e^{-\ln(19)\left[(EFL_i - EFL_{50})/(EFL_{50} - EFL_{95})\right]}\right), \quad (3)$$

where EFL_i = the EFL of fish i; and
EFL_{50} and EFL_{95} = the EFLs at which 50% and 95% of the assemblage reached maturity.

EFL_{50} and EFL_{95} were estimated by maximizing a log-likelihood function, and by assuming a binomial error distribution with AD Model Builder (Fournier, 2000).

Batch fecundity

Annual fecundity was estimated from the number of oocytes released per spawning (batch fecundity), the percentage of females spawning per day (spawning fraction), and the duration of the spawning season (Hunter and Macewicz, 2003; Murua et al., 2003) because blue marlin spawn multiple times during the season and have indeterminant fecundity (see *Results* section). There are two methods for estimating the batch fecundity: 1) the hydrated oocyte method; and 2) the oocyte size-frequency method (see Hunter et al., 1985). The oocyte size-frequency method was employed because the sample size for the batch fecundity estimates from the hydrated oocyte method is insufficient for later analysis of the relationship between batch fecundity and EFL or RW (fewer samples have migratory nucleus and hydrated oocytes been observed). The most advanced yolked, migratory nucleus, and hydrated oocytes that were destined to be spawned were identified for individuals on the basis of size-frequency distributions of whole oocytes (larger than 1000 microns). Batch fecundity was then back-calculated gravimetrically by the product of gonad weight and oocyte density, where oocyte density was the mean number of oocytes per gram of three ovarian tissues with no early postovulatory follicles (Hunter et al., 1985). Relative batch fecundity was expressed as batch fecundity divided by the round weight of the fish.

Spawning frequency

The spawning frequency of blue marlin was estimated indirectly by the inverse of the spawning fraction because direct monitoring of the spawning frequency of pelagic fish during the spawning season is difficult. The spawning fraction was calculated as the proportion of fish that spawned each day during the spawning season. There are two approaches to determine this average (Hunter and Macewicz, 1985): 1) the postovulatory follicle method; and 2) the hydrated oocyte method. Indirect methods for estimating spawning frequency are based on four assumptions: 1) females are spawning asynchronously throughout the spawning season; 2) fishes do not immigrate to or emigrate from the spawning ground; 3) the POFs of blue marlin are histologically detectable for no more than 24 hours or all hydrated oocytes are spawned in less than 24 hours (as observed for yellowfin tuna; Schaefer, 1996); and 4) the POFs do not degenerate continuously if a fish is caught and put into the refrigerator immediately.

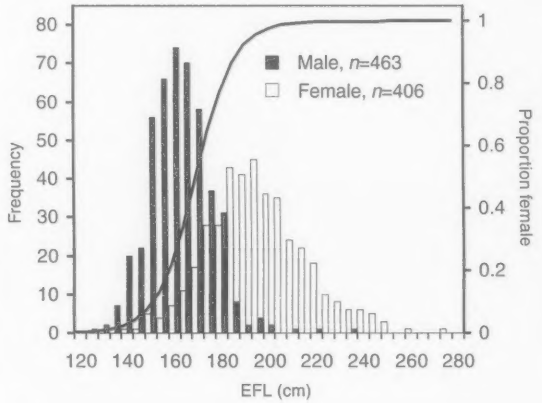

Figure 1

Length-frequency distribution of blue marlin (*Makaira nigricans*) sampled at the Tungkang fish market between September 2000 and December 2001. The solid line is the fit of a logistic curve to the proportion of females as a function of eye-to-fork length (EFL).

Results

Gonad samples, size distribution, and sex ratios

No significant differences in MOD were found between each lobe or layer within each individual blue marlin (split-plot ANOVA; F=0.41, df=1 and 52, P>0.05; F=1.30, df=2 and 51; P>0.05), although there were differences in MOD between the ovaries (F=49.97, df=2 and 52, P<0.05). Similar results were found in the analysis of oocyte number. Thus the gonad samples were collected from the random location of ovaries throughout this study (most from the posterior portion). Of the 1001 sampled fish, 406 were female (size range: 124.1–275 cm EFL), 463 were male (size range: 121–232 cm EFL), and sex could not be determined for the remaining 132 fish. Most of the sampled fish were between 140 and 220 cm EFL (Fig. 1). Blue marlin exhibit sexual dimorphism in growth. Specifically, almost all sampled blue marlin larger than 180 cm EFL were female (Fig. 1). The relationship between the female proportion (P_f) and *EFL* can be described by the logistic function (Fig. 1):

$$P_f = 1/\left(1+e^{-\ln(19)\left[(EFL-175.16)/23.59\right]}\right).$$

The sex ratio for the entire study deviated from the expected 1:1 ratio (χ^2=4.93, df=1, P<0.01) and varied among months (more males in the September 2000 [0.32], October 2000 [0.23], and May 2001 [0.31] collections, and more females in the November 2001 collection [0.67]).

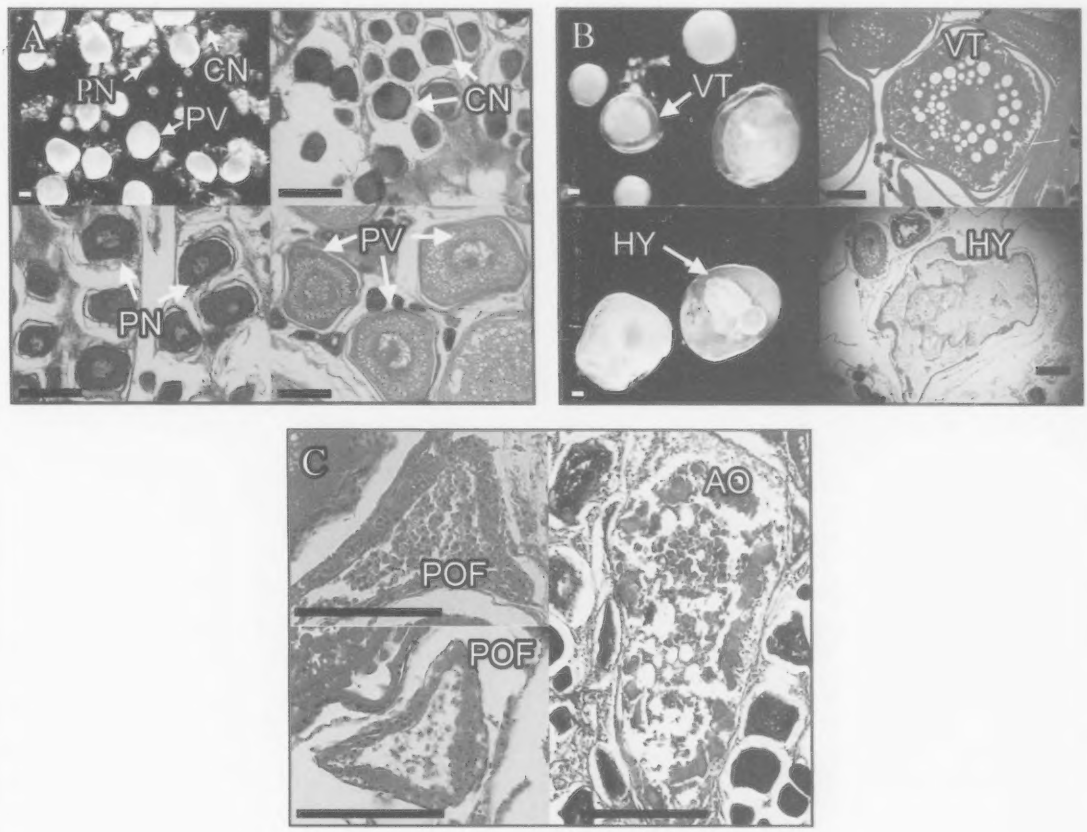

Figure 2

Micro-images of whole oocytes and sectioned oocytes of blue marlin (*Makaira nigricans*) from dissecting and light microscopes, respectively. (**A**) CN, chromatin nucleolar oocytes; PN, perinucleolar oocytes; PV, previtellogenic oocytes; (**B**) VT, vitellogenic oocytes; HY, hydrated oocytes; (**C**) POF, postovulatory follicles; AO, atretic oocyte. Scale bars: 100 microns.

Oogenesis and ovarian development

The most advanced group of oocytes for each ovary were classified as 1) unyolked—oocytes that had not begun vitellogenesis (chromatin nucleolar oocytes and perinucleolar oocytes, Fig. 2A); 2) early yolked—oocytes in the early vitellogenic stage (previtellogenic oocytes, Fig. 2A); 3) advanced yolked—oocytes in an advanced vitellogenic stage (vitellogenic oocytes, Fig. 2B); and 4) hydrated—unovulated hydrated oocyte stage (hydrated oocytes, Fig. 2B). Furthermore, the presence or absence of postovulatory follicles and atresia of yolked oocytes were identified and recorded for the determination of ovarian maturation (Fig. 2C).

The ovaries of 394 females could be staged into six ovarian development stages based on microscopic characteristics and the most advanced group of oocytes (Fig. 2 and Table 1). Ovaries were classified as immature (OM1) if they were packed with unyolked oocytes, maturing (OM2) when the unyolked oocytes were growing to early yolked oocytes, and mature (OM3) if they contained advanced yolked oocytes, but did not exhibit atresia. Ovaries that contained a migratory nucleus and hydrated oocytes, but for which no POFs were found, or for which there were unovulated hydrated oocytes and POFs, were classified as spawning-spawned (OM4). Ovaries with early yolked, advanced yolked, and atretic oocytes were assigned to the recovery stage (OM5). For resting ovaries (OM6), the unyolked oocytes were not packed as orderly as those in immature ovaries, and a few atretic oocytes were observed occasionally.

Table 1

Classification of ovary maturity stage, macroscopic and microscopic descriptions, gonadosomatic index (GSI), and mean diameter of the most advanced group of oocytes (MAGO) of ovary maturity stages of the blue marlin *Makaira nigricans*. n_h is the number of ovaries sampled for histological examination, and n_d is the number of samples for which the mean diameter of MAGO was estimated.

Ovary maturity stage	Macroscopic characteristics	Microscopic characteristics	GSI (mean ± standard error)	Mean diameter (± standard error) of MAGO
Immature (OM1)	Ovaries were solid or resilient, pinkish, with smooth streamline shape.	Ovaries are packed with unyolked or perinucleolar oocytes (Fig. 2A). No vitellogenic oocytes.	0.26 ±0.19 (n_h=66)	43.92 ±27.08 (n_d=15)
Maturing (OM2)	Ovaries became yellowish or orange with the ova developing. Oocytes were visible by eye.	Early yolked or previtellogenic oocytes were present (Fig. 2A). Some advance yolked oocytes were also observed.	0.56 ±0.24 (n_h=24)	83.91 ±53.85 (n_d=17)
Mature (OM3)	Ovary became firm and large, and the color turned orange or reddish. The surface of ovary was vascularized and translucent.	The MAGO was advanced yolked oocytes (vitellogenic oocytes, Fig. 2B). Unyolked, early yolked oocytes were also observed. No atresia of yolked oocytes were observed.	0.98 ±0.34 (n_h=31)	314.19 ±73.83 (n_d=30)
Spawning–spawned (OM4)	Ovaries reached the maximum size before spawning, and the hydrated oocytes were visible by eye. After spawning, the ovaries were flaccid. The wall of the ovary was thick.	The MAGO of the spawning fish were composed of the migratory nucleus and hydrated oocytes (Fig. 2B). Unspawned hydrated oocytes and the postovulatory follicles (Fig. 2C) were observed for spawned fish. Some early yolked oocytes were present.	4.65 ±2.78 (n_h=45)	759.01 ±173.68 (n_d=45)
Recovery (OM5)	Ovaries become small and smooth. A few yolked oocytes and hydrated oocytes were still visible.	The MAGO were composed of unyolked oocytes. Some of the yolked and the atresia of advanced yolked oocytes were present.	3.13 ±1.12 (n_h=101)	729.30 ±135.28 (n_d=31)
Resting (OM6)	Ovaries were flaccid and threadlike. Oocytes could not be observed by the naked-eye.	No vitellogenic oocytes were observed, and unyolked oocytes appeared loosely in the lumen of the ovary. A few atretic yolked oocytes (Fig. 2C) were observed.	0.98 ±0.5 (n_h=127)	304.92 ±123.37 (n_d=52)

Spermatogenesis and testicular development

The testes of 442 males could be staged into five testicular development stages by microscopic characteristics and the composition of germ cells. The five cellular stages were based on 1) spermatogonia (Fig. 3A); 2) primary spermatocytes (Fig. 3B); 3) secondary spermatocytes (Fig. 3B); 4) spermatid (Fig. 3C); and 5) spermatozoa (Fig. 3C). Testes were classified as immature (TM1) if they contained spermatogonia and primary spermatocytes, and no spermiogenesis was observed. The testes were regarded as maturing (TM2) when they contained spermatogonia, secondary spermatocytes, spermatid, and spermatozoa (<50% of total number). Testes were staged as mature (TM3) if the lobular lumens contained more than 50% spermatozoa, and the vas deferens was full of spermatozoa. Males were classified as having spawned (TM4) if the numbers of spermatozoa were decreasing in the lobular lumens and a few unspawned spermatozoa were observed in the vas deferens. Resting testes (TM5) were similar to immature testes, although a few unspawned spermatozoa were observed in the vas deferens.

Spawning season

GSI (gonadosomatic index) was relatively high between May and September for females, and between April and September for males (Fig. 4; boxplots). The condition factor, an index reflecting the interaction between biotic and abiotic factors on physiological condition, exhibited a pattern that was roughly the inverse of that of the GSI of males (Fig. 4; points-lines). There was a significant relationship between the MOD (mean oocyte diameter) and the GSI: MOD = 291.80+327.90 Ln(GSI) (r^2=0.79, n=191), and we propose that MOD

is a valid index for determining reproductive activity. Few ovaries containing vitellogenic oocytes (excluding the samples with atretic oocytes) were observed in January, February, and November. However, there was evidence for yolk accumulation between March and September based on the monthly variation of the percentage of vitellogenic ovaries and the MOD (Fig. 5). Mature ovaries were first seen in March, and females with spawning stage ovaries were observed from May to September (Fig. 6A). Postspawning females (i.e., the recovery stage) were observed from May to December. For males, mature testes were observed throughout the sampling period, and males with spawned testes were observed from March to December (Fig. 6B). The information in Figures 4, 5, and 6 together imply that the major spawning season for blue marlin in western Pacific is from May to September.

Spawning pattern

That oocytes of various developmental stages were present at the same time in an ovary was based on histological examination and is also evident from Figure 7, which shows that there were several modes in the distributions of oocyte diameter. There was a single distribution mode for diameters of early-stage oocytes: 25–35 μm for chromatin nuclear oocytes and 45–165 μm for perinuclear oocytes of immature fish. Two modes (corresponding to CN and PN) were evident in the oocyte distribution for resting fish (Fig. 7B). The number of previtellogenic oocytes (PV; 190–330 μm) began increasing with the development of the ovary for maturing fish (Fig. 7C). Vitellogenic oocytes (VT; 250–800 μm) and hydrated oocytes (800–1200 μm) appeared in the oocyte distribution when fish matured, and ocytes of several stages were present in the oocyte diameter distribution for these fish (Fig. 7D). However, there was a gap at an oocyte diameter at roughly 1000 μm. This gap indicates that oocytes larger than this size may be spawned soon (Fig. 7D). Modes corresponding to vitellogenic and unovulated hydrated oocytes were observed with the appearance of postovulatory follicles after spawning (Fig. 7E). PNs were most abundant for fish in the recovering stage, although there were also some PVs and VTs in their ovaries (Fig. 7F). Overall, Figure 7 indicates that oocytes grew in an asynchronous manner and that individual female blue marlin spawn multiple times during the spawning season.

Size-at-maturity

Maturity ogives were estimated for females and males caught during the spawning season (May to September). The relationship between the fraction mature and size can be described by a logistic curve with lengths at 50% and 95% maturity (EFL_{50} and EFL_{95}) of 179.76 ±1.01 cm EFL (estimate ±standard error, SE) and 194.2 ±1.01 cm EFL (n=394), respectively. For males, EFL_{50} and EFL_{95} were 130 ±1 and 130.13 ±46.56 cm EFL (n=442, Fig. 8).

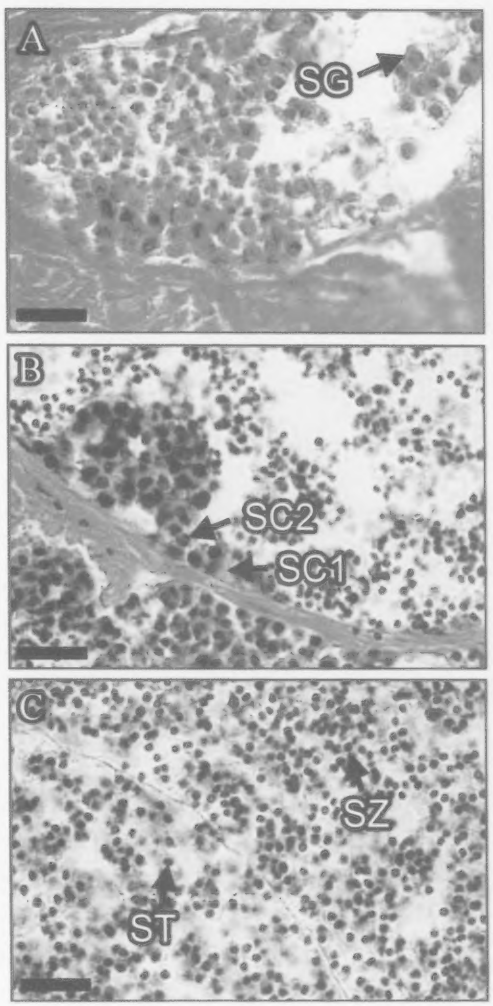

Figure 3

Developmental stages and spermatogenic cells observed in blue marlin (*Makaira nigricans*) testes. (**A**) SG, spermatogonia; (**B**) SC1, primary spermatocytes; SC2, secondary spermatocytes; (**C**) ST, spermatids; SZ, spermatozoa. Scale bars: 100 μm.

Batch fecundity

There was a clear gap in the oocyte distribution for mature ovaries at 1000 μm (Fig. 7D), and the oocytes of most advanced mode (composed of the most advanced yolked, migratory nucleus, and nonovulated hydrated oocytes) were considered to be those of the spawning batch. Batch fecundity, estimated for the 26 mature ovaries with no early postovulatory follicles, ranged from 2.11 to 13.50 million eggs (6.94 ±0.54; mean ±SE).

The relationships between batch fecundity (BF) and EFL, and between BF and RW were $BF = 3.29 \times 10^{-12} EFL^{5.31}$ (r^2=0.70; Fig. 9A) and $BF = 1.59 \times 10^{-3} RW^{1.73}$ (r^2=0.67; Fig. 9B), respectively. The batch fecundity of blue marlin was size related, and fecundity increased nonlinearly with body size. The relative fecundity of blue marlin ranged from 115 to 25 mature eggs per gram of female body weight (55.45 ±3.36).

Spawning frequency

The spawning fractions were estimated as the proportion of mature females with POFs or hydrated oocytes during the spawning season (May to September, 150 days). There was no significant difference in the spawning fraction among months within the spawning season for the postovulatory follicle and hydrated oocyte methods (χ^2=3.62, df=4, P>0.05; χ^2=1.97, df=4, P>0.05). There was also no significant difference in the spawning fraction between different size groups (<200 and ≥200 cm EFL) for these methods (χ^2=0.29, df=1, P>0.05; χ^2=0.03, df=1, P>0.05). Finally, there was no difference between the two methods in terms of the monthly spawning fraction (chi-squared independence tests; χ^2=3.54, df=4, P>0.05). The spawning fraction of mature females based on the postovulatory method was 0.41 (n=164; data combined over months), which indicates that each female would have spawned on average once every 2.4 days, or 62 times during the spawning season (Table 2). In contrast, 34% of the mature females had hydrated oocytes during the spawning season, which is equivalent to a mean spawning interval of 2.9 days, or a spawning frequency of 51 times (Table 2). Annual fecundity was estimated as 120–769 million eggs based on the product of batch fecundity and the average spawning frequency from the two methods (57 times).

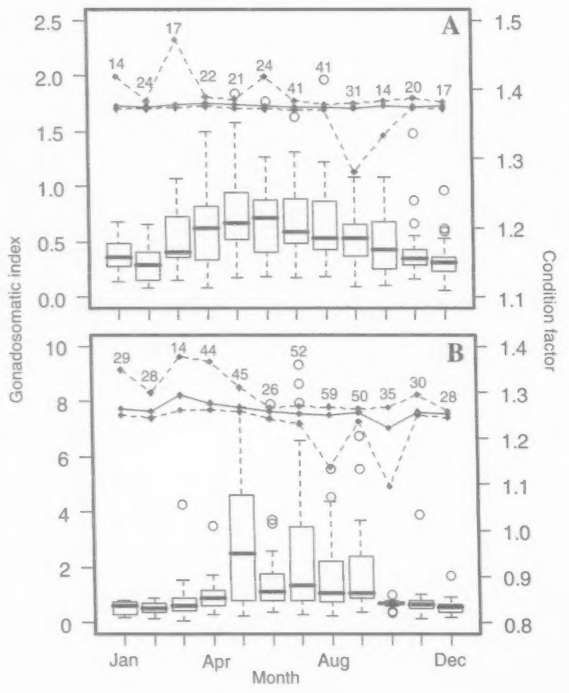

Figure 4

Monthly variation in the gonadosomatic index (GSI; boxplots, left axis) and condition factor (mean=solid-line diamonds, and interquartile range=dashed-line diamonds, right axis) for (**A**) female and (**B**) male blue marlin (*Makaira nigricans*) collected at the Tungkang fish market between September 2000 and December 2001 (monthly data combined over years). The numbers indicate how many individuals were examined.

Discussion

Size distribution and sex ratio

The sizes of blue marlin caught by Taiwanese offshore longliners in the western Pacific Ocean were primarily between 140 and 220 cm EFL. However, blue marlin are sexually dimorphic; animals 170 cm and less are generally males and those larger than 180 cm are generally female. Shung (1975) obtained similar results for blue marlin in the South China Sea (Pratas Islands). Reproductively active male blue marlin in the eastern Pacific Ocean are often smaller than 220 cm EFL, but all animals 230 cm EFL and larger are female (Kume and Joseph, 1969). Several billfish species, such as sailfish (Chiang et al., 2006b), blue marlin (Wilson et al., 1991) and swordfish (DeMartini et al., 2000; Wang et al., 2003) have been shown to exhibit sexually dimorphic growth. Several hypotheses have been proposed to explain this, including 1) sex change or a sex-specific mortality rate (deSylva, 1974); 2) sex-specific growth rates (Wilson et al., 1991; Sun et al., 2002); and 3) sex-specific natural mortality rates (Skillman and Yong, 1976; Sun et al., 2005). Histological analyses indicated that sex-change does not occur in blue marlin. Furthermore, Chen (2001) showed that growth of blue marlin differs between females and males. Thus, this dimorphism is most likely caused by sex-specific growth and mortality rates. However, there is a need to examine the degree to which sexual dimorphism is due to each of these latter two factors. Departure from a 1:1 sex ratio is not expected for most fish species even though females dominate the large size classes. The sample was male-biased for the nonspawning season. However, the sex ratio during the putative spawning season (May–September) was more balanced (χ^2=0.07, df=1, P>0.05). This

Table 2

Spawning fraction and spawning frequency during the spawning season determined using the postovulatory follicle (POF) and hydrated (HY) oocyte methods for female blue marlin (*Makaira nigricans*) in the western Pacific Ocean. EFL is the eye-to-fork length measurement.

		POF method			HY method		
Month	*n*	Ovary with POFs	Spawning fraction	Spawning frequency	Ovary with HY oocytes	Spawning fraction	Spawning frequency
May	20	5	0.25	4.00	8	0.40	2.50
Jun	26	12	0.46	2.17	6	0.23	4.33
Jul	39	17	0.44	2.29	17	0.44	2.29
Aug	46	25	0.54	1.84	16	0.35	2.88
Sep	33	9	0.27	3.67	9	0.27	3.67
Size							
<200 cm EFL	86	39	0.45	2.21	28	0.33	3.07
≥200 cm EFL	78	29	0.37	2.69	28	0.36	2.79
Total	164	68	0.41	2.41	56	0.34	2.93

may imply that mature females migrate to the spawning grounds during the spawning season. The exact spawning area of blue marlin in the western Pacific Ocean needs to be identified in the future in order to fully evaluate this hypothesis.

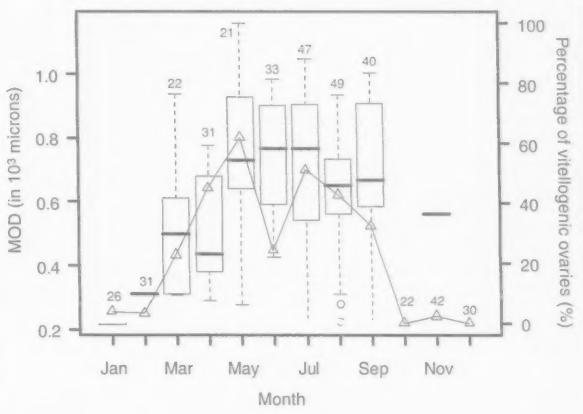

Figure 5

Monthly variation in the mean diameter of the most advanced group of oocytes (MOD; boxplots, left axis) and the percentage of vitellogenic ovaries (excluding the samples with atretic oocytes) (triangles, right axis) for blue marlin (*Makaira nigricans*) collected at the Tungkang fish market between September 2000 and December 2001 (monthly data combined over years). The numbers indicate how many ovaries were examined.

Maturity classification and gonad maturation

Misclassification of whether a female is mature will contribute error to the estimation of the maturity ogive, spawning season, batch fecundity and spawning frequency, and hence to uncertainty when estimating size-at-maturity and egg production. In this study, the use of histological techniques to study gonad maturation provided a more precise outcome than have traditional macroscopic techniques. The spawning season for females was estimated by using GSI and histology and by identifying oogenesis as well as the temporal variation of the mean diameter of the most advanced group of oocytes. The size-frequency distributions of whole oocytes (cross calibrated with histological characteristics) for the different maturation stages provided a fuller understanding of the dynamics of ovarian maturation and the spawning pattern of a fish that produces multiple batches. The size-frequency distributions of whole oocytes, which can be constructed by using a dissecting microscope, can serve as a quick way to determine the ovarian development stage when histological data are not available. The condition factor is usually related to fish health, and a roughly inverse pattern between the temporal variation of the GSI and condition factor was found in male blue marlin, which may imply a lower feeding activity during the spawning season as has been argued for some other pelagic migratory fishes (i.e., school mackerel, Begg and Hopper, 1997).

Size-at-maturity

The EFL_{50} for female blue marlin was estimated to be 179.76 cm, and the smallest size at which any female was mature was 157.8 cm EFL. For males, there was considerable uncertainty regarding EFL_{50} owing to a

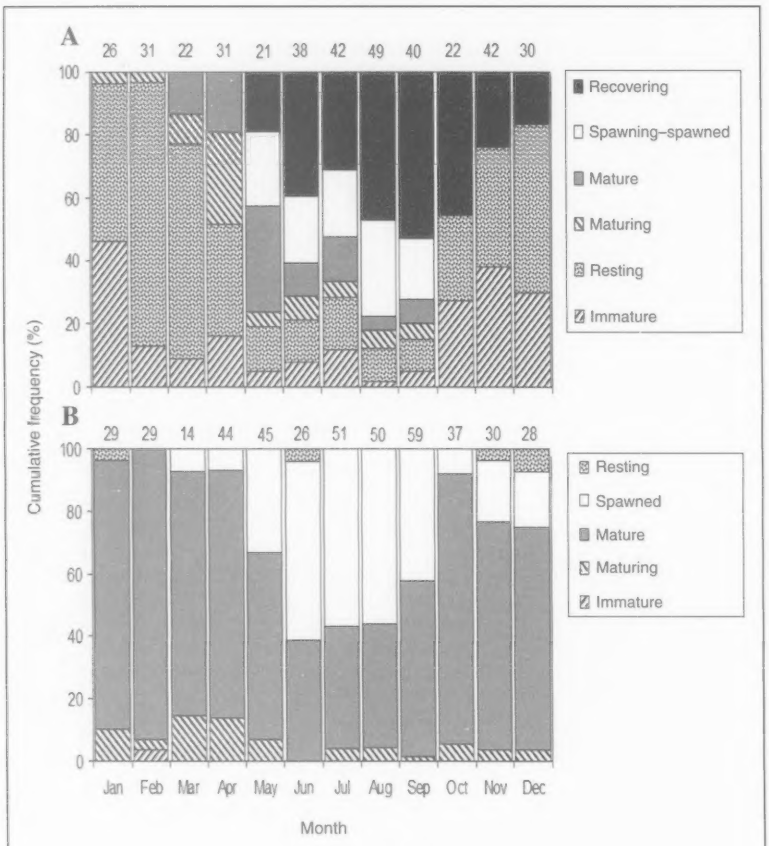

Figure 6

Percentage of blue marlin (*Makaira nigricans*) collected from the Tungkang fish market between September 2000 and December 2001 in each gonad development stage by sex and month: (**A**) females, and (**B**) males (monthly data combined over years). The numbers indicate how many animals were examined.

lack of samples in the size range when male blue marlin are maturing (i.e., within the transition from immature to mature) (Fig. 8). However, male blue marlin larger than 131 cm EFL (size-at-onset-of-maturity) were all mature. The size-at-maturity of blue marlin appears to vary across different regions of the Pacific Ocean. For example, the size-at-onset-of-maturity for male blue marlin was roughly 130–140 cm EFL in the western Pacific Ocean (Nakamura, 1985), and females 171–180 cm EFL have GSI values larger than three (i.e., have reached sexual maturation) in the eastern Pacific Ocean (Uosaki and Bayliff, 1999). This variation among areas may be due to environmental or genetic effects or simply sampling error. In this study, EFL_{50} for females was based on large sample sizes and a broad size range of fish collected throughout the spawning season, and maturity was determined by histology. Thus, the results of this study should provide an accurate representation of the size-at-maturity of blue marlin in the western Pacific Ocean.

Spawning season

Blue marlin have indeterminate fecundity, exhibit an asynchronous oocyte development pattern, and spawn multiple times during the spawning season (Hunter and Macewicz, 2003; Murua et al., 2003). The major spawning season seems to be from May to September based on MOD, the GSI values, and staging of ovaries. Shung (1975) indicated that blue marlin spawn between February and November (with high activity in June and September) in the South China Sea, and Hopper (1990) argued that blue marlin spawn primarily between May and September in Hawaiian waters. Kume and Joseph (1969) indicated that blue marlin spawn from December to January in the South Pacific Ocean. In the Atlantic

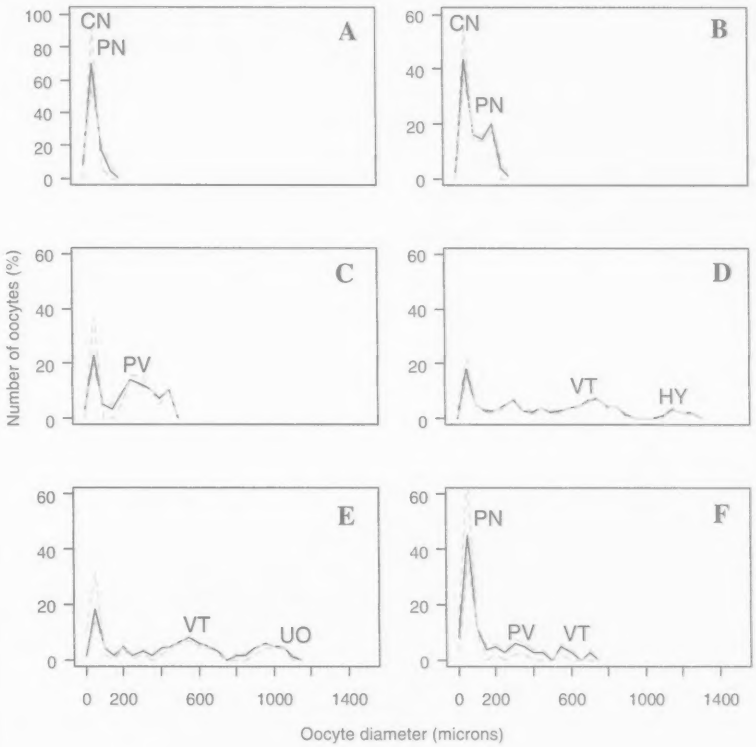

Figure 7

Frequency distributions (percentages) of oocyte diameter (black solid lines) with 95% bootstrapped confidence intervals (gray dashed lines) for six ovarian developing stages of blue marlin (*Makaira nigricans*): (**A**) immature; (**B**) resting; (**C**) maturing; (**D**) mature; (**E**) spawning–spawned; and (**F**) recovering. CN, chromatin nucleolar oocytes; PN, perinucleolar oocytes; PV, previtellogenic oocytes; VT, vitellogenic oocytes; HY, hydrated oocytes; UO, unovulated hydrated oocytes.

Ocean, the spawning season for blue marlin is May through September, but most activity is between July and August according to data collected near Puerto Rico (Erdman, 1968), although Yeo (1978) indicated that blue marlin spawn at temperatures of 26–29°C from April to September in the western North Atlantic. These estimates of spawning season would indicate that blue marlin have an extended spawning season and may be more reproductively active during summer, perhaps because of higher temperatures at that time.

Batch fecundity

It has been argued that more accurate estimates of batch fecundity can be obtained by using only oocytes in the migratory nucleus and hydrated stages (sailfish, *Istiophorus platypterus*; Chiang et al., 2006a). Unfortunately, few ovary samples with migratory nuclei or hydrated oocytes were observed in our study. However, the oocyte distributions exhibited clear modes, including oocytes at sizes that are ready to be spawned. The oocyte size-frequency method usually yields results similar to those based on counts of hydrated oocytes if females with highly advanced oocytes are used (Hunter et al., 1985). In this study, the gonad tissues were collected and preserved for further examination. Batch fecundity is usually back-calculated gravimetrically as the product of the oocyte density per gram of the preserved tissue and the total fresh weight of the ovary. However, Ramon and Bartoo (1997) indicated that preserved ovaries of mature albacore tuna (*Thunnus alalunga*) lost an average of 2% of their fresh weight. The effect of preservation on weight lost may bias the estimation of batch fecundity by the gravimetric method and may also bias identification of the most advanced-stage oocytes. Thus, gonad weights for fresh

and preserved samples should be compared in order to more fully evaluate the gravimetric and other (e.g., volumetric) methods.

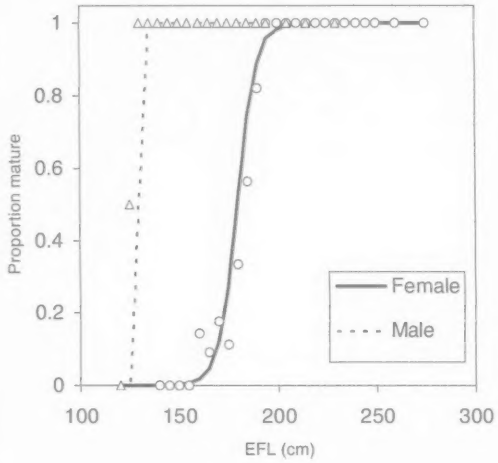

Figure 8

Maturity-at-length (eye-to-fork length [EFL]) relationships for female (solid lines) and male (dashed lines) blue marlin (*Makaira nigricans*). The open circles and triangles represent the observed proportions mature-at-age for females and males respectively (aggregated to 5 cm intervals).

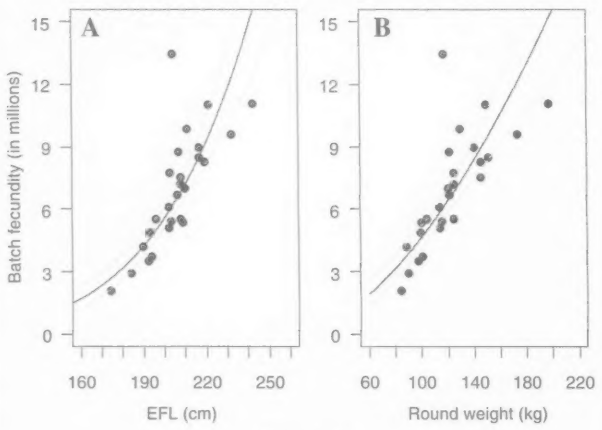

Figure 9

Batch fecundity as a function of (**A**) eye-to-fork length (EFL), and (**B**) round weight for blue marlin (*Makaira nigricans*) collected from the Tungkang fish market between September 2000 and December 2001. The solid lines denote the predicted fecundity, and the dashed lines the 95% confidence intervals for the mean relationship.

Individual estimates of batch fecundity ranged from 2.11 to 13.50 million eggs (6.94 ±0.54; size range 174–242 cm EFL). Batch fecundity of blue marlin is estimated to be larger than that of the black marlin (*Makaira indica*) in the waters off Taiwan (0.32–3.2 million eggs; Liu, 2007), than that of sailfish in eastern Taiwan waters (0.2–2.48 million eggs; Chiang et al., 2006a), and that of swordfish (*Xiphias gladius*) in the waters off eastern Australia (1.16–2.50 million eggs; Young et al., 2003).

Spawning frequency

We assumed that the hydrated oocytes of blue marlin were spawned in less than 24 hours (the hydrated oocyte method) and that the POFs were detectable for no more than 24 hours (the postovulatory follicle method), given observations for other pelagic fish (yellowfin tuna, *Thunnus albacares*; Schaefer, 1996). However, these assumptions need to be verified in the future. The mean time between consecutive spawning events was 2.4 days based on the postovulatory follicle (POF) method (2.9 days based on the hydrated oocyte method). There was no significant difference in spawning fraction among months, which indicates that females were spawning asynchronously throughout the spawning season. Furthermore, there is no relationship between the monthly spawning fraction and the two methods, which may indicate that the estimates of the spawning frequency are accurate. However, there are two caveats which need to be examined when applying the POF method. First, the degeneration of POFs varies among species and may be influenced by the preferred spawning temperature of a species (Chiang et al., 2006a). Second, the fish we collected from the fish market may have been caught a few days earlier. Because of that, it is possible that the POFs degenerated before we obtained the fish and therefore the fraction of ovaries with POFs may have been underestimated.

Conclusion and recommendations

This study provides reproductive parameters and their associated uncertainty as inputs for use in stock assessments of blue marlin in the western Pacific Ocean. The analyses were based on large samples from Taiwanese offshore longliners that cover broad areas in the western Pacific Ocean. Consequently, the estimates should be reliable. However, collecting by ships a greater number of specimens over the entire stock's spatial distribution (especially for hydrated ovaries, and testes during the spawning season) is recommended so that estimates of male size-at-maturity and egg production will be more robust. The spatial variation in some of the reproductive

parameters could be explored further by using data collected over a broader spatial domain and hence an exploration could be conducted to determine whether this variation is related to abiotic or biotic factors. We recommend that the methods used in this study be applied to estimate reproductive parameters for billfish and other pelagic fish.

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Spatial and temporal distribution and the potential for estuarine interactions between wild and hatchery chum salmon (*Oncorhynchus keta*) in Taku Inlet, Alaska

Carl Reese (contact author)[1,2]
Nicola Hillgruber[2]
Molly Sturdevant[3]
Alex Wertheimer[3]
William Smoker[2]
Rick Focht[4]

Email address for contact author: carl.reese@alaska.gov

[1] Alaska Department of Environmental Conservation
410 Willoughby Avenue
Juneau, Alaska 99801

[2] School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
17101 Point Lena Loop Road
Juneau, Alaska 99801

[3] Auke Bay Laboratories
Alaska Fisheries Science Center
National Marine Fisheries Service
17109 Point Lena Loop Road
Juneau, Alaska 99801

[4] Douglas Island Pink and Chum, Inc.
2697 Channel Drive
Juneau, Alaska 99801

Abstract—We investigated estuarine spatial and temporal overlap of wild and marked hatchery chum salmon (*Oncorhynchus keta*) fry; the latter included two distinct size groups released near the Taku River estuary (Taku Inlet) in Southeast Alaska (early May releases of ~ 1.9 g and late May releases of ~ 3.9 g wet weight). Our objectives were to compare abundance, body size, and condition of wild chum salmon fry and hatchery chum salmon fry raised under early and late rearing strategies in different habitats of Taku Inlet and to document environmental factors that could potentially explain the distribution, size, and abundance of these chum salmon fry. We used a sampling design stratified into inner and outer inlet and neritic and littoral habitats. Hatchery fry were rare in the inner estuary in both years but outnumbered wild fry 20:1 in the outer estuary. Hatchery fry were significantly larger than wild fry in both littoral and neritic samples. Abundances of wild and hatchery fry were positively correlated in the outer inlet, indicating the formation of mixed schools of hatchery and wild fry. Spatial and temporal overlap was greatest between wild and early hatchery fry in the outer inlet in both habitats. The early hatchery release coincided with peak abundances of wild fry in the outer inlet, and the distribution of wild and early hatchery fry overlapped for about three weeks. Our results demonstrate that the timing of release of hatchery fry may affect interactions with wild fry.

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Salmonids have been reared and released from hatcheries since the nineteenth century (Olla et al., 1998) and enhancement facilities now exist worldwide (e.g., Mahnken et al., 1998; Kaeriyama, 1999; Zaporohzets and Zaporohzets, 2004). Many authors have expressed concern that large-scale salmonid hatcheries may diminish fitness and productivity of wild populations (Hilborn and Eggers, 2000; Einum and Fleming, 2001; Zaporozhets and Zaporozhets, 2004; Araki et al., 2007). Others have shown that hatchery releases support the recovery of declining populations (Heard et al., 1995; Brannon et al., 2004) and can enhance fisheries without impacting wild stocks (e.g., Bachen and Linley, 1995; Heard et al., 1995; Wertheimer, 1997). Hatchery salmonids can impact wild fish in a number of ways, such as through spawning interactions, genetic interactions, inducing overharvesting, and by trophic or behavioral interactions during estuarine and oceanic life-history phases (Levings et al., 1986; Bigler et al., 1996; Olla et al., 1998). Hatchery salmonids can also impact wild fry populations by either attracting predators that selectively eat smaller wild fry (Hargreaves and LeBrasseur 1986; Wertheimer and Thrower, 2007; Duffy and Beauchamp, 2008) or hatchery salmonids can benefit wild populations by buffering wild fry from predators (Willette et al., 2001; Briscoe et al., 2005). The potential for hatchery fish to affect wild fish increases with the degree of spatial and temporal overlap between fishes of similar life-history stages. Hatcheries often employ different rearing and release strategies to reduce these impacts, such as releasing fry away from streams that produce wild fish (Bachen and Linley, 1995; Heard et al., 1995) or releasing

fry later at a larger size to reduce overlap with wild fry, thus decreasing nearshore residency of hatchery fish and increasing survival of wild fish (Kaeriyama, 1999; Wertheimer and Thrower, 2007).

Douglas Island Pink and Chum (DIPAC), an enhancement hatchery near Juneau, Alaska, uses two different release strategies simultaneously for chum salmon (*Oncorhynchus keta*). These strategies provide an opportunity to study spatial and temporal overlap of wild fry with both hatchery fry that are released early at a smaller body size and those released later and at a larger size. Spatial and temporal overlap between these hatchery fry and wild fry in the Taku River estuary is likely because of the proximity of the release sites, and thus interactions are possible. Hatchery juveniles are released near Taku Inlet in the spring when wild fry are most abundant. The DIPAC hatchery has placed unique thermal marks (Orsi et al., 2004) on all groups of chum salmon fry released since 1991, and has used distinct marks by location for smaller earlier groups and for larger later groups. DIPAC is thought to have more than doubled the annual adult chum salmon catch in the region based on releases of over 100 million fry near Juneau each spring; over the past 5 years annual catches of returning adult chum salmon that were released from DIPAC have averaged approximately 2 million fish. At the same time, independent indices of wild chum salmon abundance from the Taku River have declined significantly, raising questions about stock interactions. Catch per unit of effort (CPUE) for wild chum salmon caught in the commercial gillnet fishery in Taku Inlet is negatively related to the number of chum salmon fry released from DIPAC hatchery near Juneau during their natal year (Fig. 1; coefficient of determination from regression of ln adult catch against fry releases, r^2=0.77, P=0.01). This relationship indicates that releases of hatchery chum salmon fry may contribute to the decline of wild chum salmon.

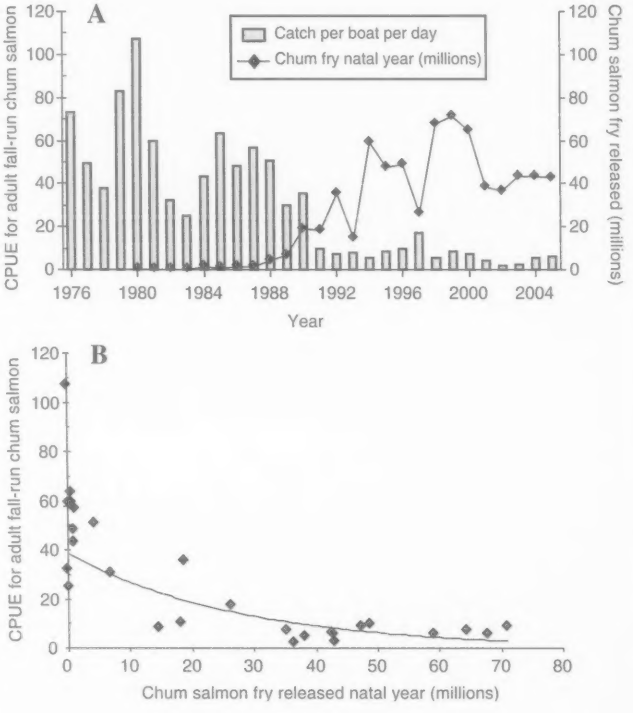
A
Catch per boat per day
Chum fry natal year (millions)
CPUE for adult fall-run chum salmon
Chum salmon fry released (millions)
Year
B
CPUE for adult fall-run chum salmon
Chum salmon fry released natal year (millions)

Figure 1

Trends in abundance of chum salmon (*Oncorhynchus keta*) near Taku Inlet, Alaska, as (**A**) annual commercial catch-per-boat-day (CPUE) of wild fall-run adults (4-year-old) and Douglas Island Pink and Chum (DIPAC) hatchery fry releases (millions of fish), and (**B**) adult chum salmon CPUE versus number of hatchery fry released near the inlet (regression of ln *CPUE*: r^2=0.77, P <0.01, df=24), 1976–2005. Fry data were shifted to four years later to match natal year to adult harvest year.

Four kinds of potential wild and hatchery interactions have been proposed, namely 1) marine phase ecological interactions, 2) spawning competition and interbreeding interactions, 3) induced overharvesting interactions, and 4) early estuarine phase ecological interactions. We considered interactions during the estuarine life-history phase as the most plausible mechanism for the decline in wild Taku River fall chum salmon runs because other salmon populations in Southeast Alaska have been stable, including Taku summer chum salmon runs, and because Pacific salmon populations are known to co-vary in response to ocean conditions (Pyper et al., 2002; Mueter et al., 2005). The estuarine phase is known to be a period of high mortality with many variables influencing juvenile salmon growth and survival, including density and competition (e.g., Simenstad et al., 1982; Willette et al., 2001; Wertheimer and Thrower, 2007; Duffy et al., 2005). Thus, the goal of this study was to investigate habitat use and migration timing of wild and hatchery chum salmon fry in Taku Inlet to evaluate the potential for wild and hatchery fish to interact in this estuary. Specifically, our objectives were 1) to compare the abundance of wild and hatchery chum salmon fry raised under early and late rearing strategies in different habitats of Taku Inlet (inner and outer inlet locations, littoral and neritic habitats); 2) to compare the body size and condition of these groups of fry in different habitats; and 3) to document environmental factors that potentially could explain the distribution and size of chum salmon fry and the abundance of hatchery fry.

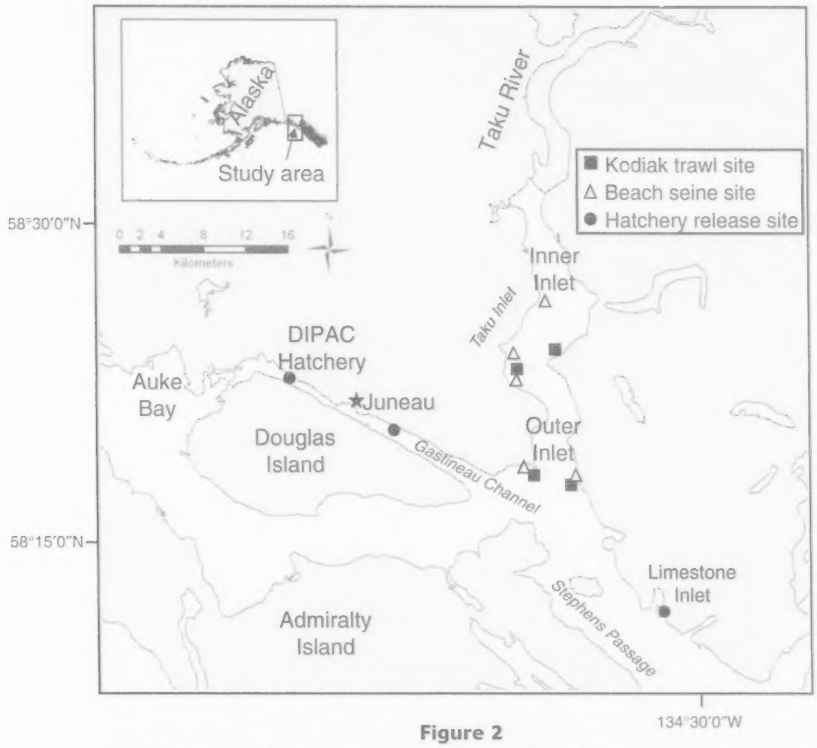

Figure 2

Sites sampled by beach seine (triangles) and Kodiak trawl (squares) in inner and outer Taku Inlet, Alaska, from late April to late June, 2004–05, and nearby hatchery release sites (circles) for chum salmon (*Oncorhynchus keta*) fry. Sampling began before the outmigration period for wild fry (late April to mid-June) and before hatchery releases of approximately 25 million "early" fry (released in early May at 1.9 g size) and 25 million "late" fry (released in late May at 3.9 g size) each year.

Background and study area

The Taku River enters the ocean 20 km south of Juneau, Alaska. About 90% of its 16,000 km^2 watershed is located in a roadless wilderness in British Columbia, Canada. Taku Inlet is a narrow fjord, 3–6 km wide by 18 km long (Fig. 2). In addition to chum salmon, the Taku River supports stable runs of sockeye (*O. nerka*), Chinook (*O. tshawytscha*), coho (*O. kisutch*), and pink salmon (*O. gorbuscha*). Migration timing of chum salmon fry in the Taku River is adapted to decreases in salinity and increases in sea surface temperature that typically occur from April through June (Meehan and Siniff, 1962; Murphy et al., 1997).

Annual production of chum salmon released by DIPAC at Limestone Inlet and Gastineau Channel near Taku Inlet increased from <1 million fry in 1982 to approximately 60 million fry in 1994 and production has generally remained above 40 million since then (Fig. 1). DIPAC has also released 50–60 million thermally marked fry annually in northern Southeast Alaska at sites distant from Taku Inlet. During this study, 26 million "early" fry were released May 7–17 at 1.9 g wet weight and 24 million "late" fry were released May 22–June 3 at 3.9 g in the vicinity of Taku Inlet in each year. These groups were produced from the same summer brood stock derived from coastal streams near Juneau and differed only in the rearing duration and the size and date at release.

Methods

Field sampling

Sampling was conducted from late April to late June, 2004–05, thus covering the outmigration period for chum salmon in Taku Inlet (Meehan and Siniff, 1962; Murphy et al., 1997). We conducted two 48-hour cruises per week, in which littoral and neritic habitats were sampled with different gear and at different times of day. We sampled littoral habitat throughout the inlet where it was feasible to use a 37-m long×3-m deep beach seine (3-mm bunt mesh) (Mortensen et al., 2000; Johnson and Thedinga,

2006). Beach gradient was 9–14% and substrate was predominantly gravel <5 cm. Three beaches were seined in the inner inlet and two in the outer inlet (Fig. 2). All five beach seine sites were sampled during daylight for a minimum of three times per week. At each beach seine site, sea surface temperature was measured with a thermometer and a water sample was collected to determine salinity.

We sampled neritic habitat with a two-boat Kodiak trawl at night (Moulton, 1997; Mortensen et al., 2000). The 6-m wide × 15-m long × 3-m deep surface trawl (3-mm codend mesh) was towed at the surface at 2 knots, 40–100 m offshore, parallel to the shoreline and at a bottom depth of 10–20 m. We trawled two nights per week at two sites in the outer inlet and two in the inner inlet (Fig. 2; 8 samples per week). Each tow lasted 10 minutes. At each trawl site, a temperature and salinity profile was taken with a Seabird SBE-19 Seacat conductivity-salinity-depth (CTD) profiler (Sea-Bird Electronics, Bellevue, WA).

Fish from both beach seine and trawl sets were processed in the same manner and fish treatment followed a protocol approved by the University of Alaska Fairbanks Animal Care and Use Committee (IACUC no. 05-19). All captured fish were anesthetized with tricaine methanesulfonate (MS-222), identified to species, and counted; nontarget species were released after identification. A maximum of 60 chum salmon per set was euthanized with excess MS-222 and either preserved in 10% formaldehyde-seawater solution or frozen for subsequent laboratory analysis. Preserved fry were transferred to 50% isopropyl to maintain the integrity of the otolith.

Laboratory processing

Each preserved or frozen fish was weighed to the nearest 1.0 mg wet weight (wt), and measured to the nearest 1.0 mm fork length (FL). Otoliths were removed and fry were identified by origin from the presence and type of thermal marks. Each year DIPAC placed a unique thermal mark on fry released near Taku Inlet: one mark for Gastineau Channel early fry, one for Gastineau Channel late fry, and one for Limestone Inlet fry(Fig. 2). Early and late hatchery fry were released from Limestone Inlet in both years but the two groups were given the same thermal mark. All fry caught from Limestone Inlet releases before release of the late hatchery fry were assumed to be early hatchery. All fry without thermal marks were assumed to be wild. Inferences about the distribution of late hatchery fry from Limestone Inlet after they were released were based on Gastineau Channel late-hatchery-fry data.

Data analysis

We analyzed data from 2004 and 2005 separately. Sampling sites were pooled by littoral or neritic habitat for inner or outer inlet locations. The inner inlet sites were closest to the mouth of the river (the source of wild fish) and the outer inlet sites were closer to the hatchery release sites (Fig. 2), and therefore there was an *a priori* expectation that hatchery fry would be more abundant in the outer inlet. The inner and outer inlets were stratified because sea surface temperature and salinity were noticeably higher in the outer inlet location and the probability of encountering hatchery fry was greater in the outer inlet. Data on early fry from both hatchery release sites were pooled for analysis for two reasons: first, early hatchery fry from Gastineau Channel and Limestone Inlet were released at almost the same time and were similar in size; and second, the fry from both release sites were commonly found on both the east and west sides of the inlet (Fig. 2). We conducted three types of analyses: 1) spatial and temporal analyses to compare the abundances of wild and hatchery chum salmon fry; 2) spatial and temporal analyses to contrast the body sizes of hatchery and wild chum salmon fry; and 3) analyses to correlate the distribution and size of wild chum fry with hatchery fry distribution, sea surface temperature, and salinity.

Spatial and temporal distribution of relative abundance

Total catch of chum salmon for each set (seine or trawl) was apportioned by hatchery origin or wild origin according to the proportion of thermally marked fry in the sample. We calculated CPUE of wild and hatchery salmon separately as the mean number of chum salmon captured per set by week in the inner or outer inlet locations (Fig. 2). We plotted CPUE as an indicator of fry abundance. The proportions of wild and hatchery fry in the catch were calculated by week through the season and plotted separately by habitat and location. We could not determine when individual wild fry entered the estuary, but hatchery fry were assumed to have resided in the area since time of release.

Spatial and temporal change in body size of hatchery and wild salmon

Mean fork length and weight of wild, early hatchery, and late hatchery chum salmon fry were plotted by week over the course of the emigration period by location and habitat. The change in the mean size of each fish stock over time was calculated as an indirect measure of apparent growth. Although we acknowledge that immigration, emigration, and size-selective mortality are confounding effects on growth, we could not account for these changing processes. We determined apparent growth rate using the slope of the regression of fork length on date caught (day of the year). Fork length of fry of all origins was plotted into four histograms per year by location and habitat. Differences in length among location (inner, outer) and habitat type (littoral, neritic) for each year were analyzed with a one-way analysis of variance (ANOVA) for wild fry. The ANOVA compared length of wild fry by location and habitat. Fork length of early hatchery fry was analyzed by using the same ANOVA procedure. We used *t*-tests to examine length

for late hatchery fry between habitat types because no late hatchery fry were caught in the inner inlet. For all these analyses, fork length was ln-transformed because some of the frequency distributions were not normally distributed.

Distribution, size, and condition of wild chum salmon fry in relation to temperature, salinity, and chum salmon abundance

Because of the small sample size of wild chum salmon in neritic trawls, analyses of the distribution and size of wild chum salmon were conducted only with data from littoral habitat. Correlation analysis and stepwise multiple regressions were used for fry in littoral habitat to determine the relationships of wild fry abundance, weight, and condition factor (defined below), with sea surface temperature, salinity, time (date), and total chum salmon abundance. Sea surface temperature and salinity were included in analyses because the behavior and distribution of outmigrating salmon fry in estuaries often reflects evolutionary adaptations to hydrographic conditions in the estuary (reviewed by Salo, 1991; Murphy et al., 1997). To account for morphological changes that occur with ontogeny, condition factor was calculated as the residual of the regression of ln weight versus ln fork length (Jakob et al., 1996). Data from sets made before the release of hatchery fry were excluded from the analysis in order to address our goal of determining if wild fish distribute themselves differently in the presence of hatchery-released fish. Separate analyses were conducted for each year, by location, and habitat. A forward-backward stepwise regression was used with an alpha of 0.10 to include variables in the equation and an alpha of 0.10 to exclude the significance of β, by using the following equation:

$$Wild\ abundance = \beta_H\ hatchery\ abundance + \beta_T\ SST + \beta_S\ salinity + \beta_d\ date + \varepsilon, \quad (1)$$

where *abundance* = ln (*catch*+1);
SST = sea surface temperature; and
date = day of the year.

We added 1 to all catches because zeros would become undefined upon transformation. The same approach was used to relate weight of wild fry to wild fry abundance, hatchery fry abundance, sea surface temperature, and salinity. The stepwise regression used the following equation:

$$Weight\ of\ wild\ fry = \beta_W\ wild\ abundance + \beta_H\ hatchery\ abundance + \beta_T\ SST + \beta_S\ salinity + \beta_d\ date + \varepsilon, \quad (2)$$

where *Weight* = *ln* weight (g).

Finally, we analyzed the relationship between condition factor of wild fry and wild abundance, hatchery abundance, sea surface temperature, and salinity using the same techniques. The stepwise multiple regression equation used to analyze condition factors of individual wild fry was the following:

$$Condition\ factor = \beta_W\ wild\ abundance + \beta_H\ hatchery\ abundance + \beta_T\ SST + \beta_S\ salinity + \beta_d\ date + \varepsilon. \quad (3)$$

No colinearity was found in these models (Sokal and Rohlf, 1995).

Results

Spatial and temporal distribution of relative abundance

Littoral habitat Hatchery fry were most abundant in littoral habitat in the week following the early hatchery releases in both years, on 10 May 2004 and on 17 May 2005 (Fig. 3, A and B). Both hatchery and wild fry were generally less abundant in 2004 than in 2005. During both years, hatchery chum salmon represented over 95% of the catch in the outer inlet, but in the inner inlet represented only 11% in 2004 and 1% in 2005 (Fig. 4). Abundance of wild and early hatchery chum salmon fry in littoral habitat declined within two weeks in late May in both the inner and outer inlet. Abundance of late hatchery fry declined within one week of their release in late May, but unlike early hatchery chum salmon, late hatchery fry were never caught by beach seine in the inner inlet.

The greatest spatial and temporal overlap among chum salmon fry in littoral habitat occurred between wild and early hatchery fry in the outer inlet. Little potential existed for wild and early hatchery chum salmon fry to interact in inner Taku Inlet because hatchery fry were rare in this location. Similarly, little chance existed for wild and late hatchery fry to interact because the latter were not observed in the inner inlet and migrated from the outer inlet within one week of their release. The early hatchery release coincided with peak abundance of wild fry in the outer inlet and the distribution of these stocks overlapped for about 3 weeks. Wild chum salmon were present in littoral habitat from the beginning of sampling on 19 April through 21 June in both years. Abundance of wild chum salmon fry peaked in the inner inlet during the week of 17 May 2004 and the week of 3 May 2005 (Fig. 3A). Abundance of wild chum salmon fry peaked in the outer inlet in the week of 17 May 2004 and 10 May 2005 (Fig. 3B).

Neritic habitat Hatchery fry were most abundant in neritic habitat in the outer inlet during the weeks of 17–31 May. Both hatchery and wild fry were generally less abundant in 2004 than in 2005. By location, hatchery chum salmon represented over 98% of the catch in the outer inlet during both years, whereas in the inner inlet they represented 93% in 2004 and 24% in 2005 (Fig. 4). Most of the hatchery chum salmon from neritic

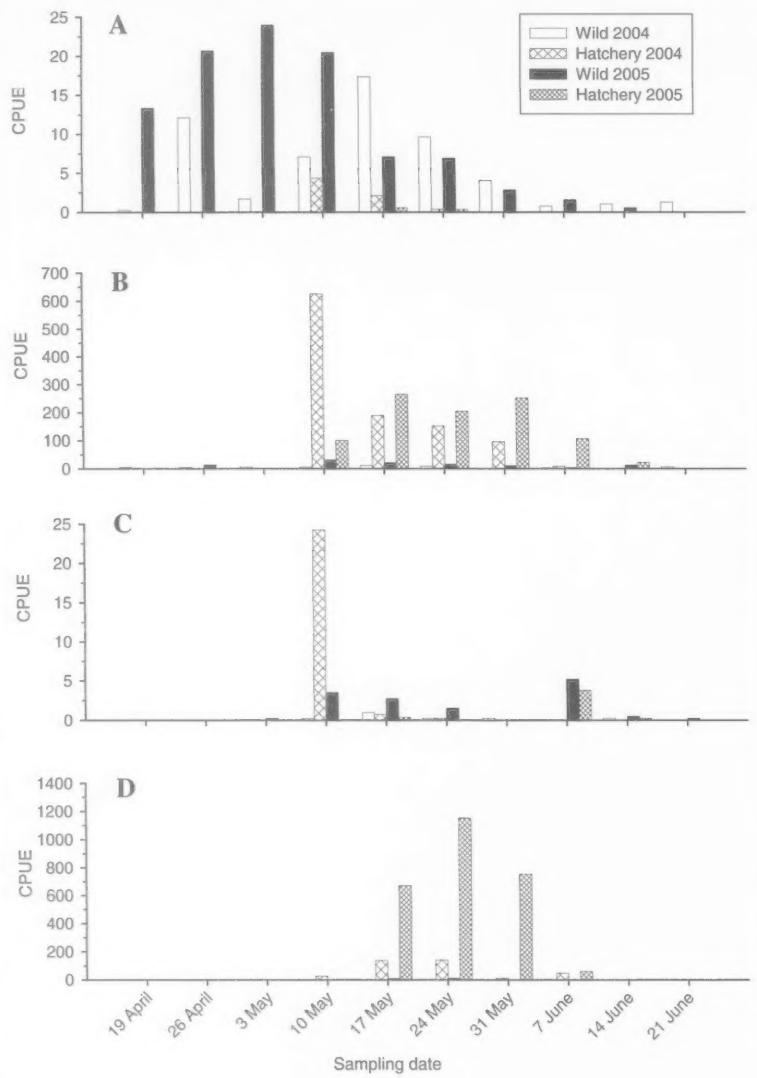

Figure 3

Mean catch-per-set (CPUE) for chum salmon (*Oncorhynchus keta*) fry in Taku Inlet, Alaska, by week during the outmigration period for wild fry (late April to mid-June): (**A**) littoral habitat, inner inlet; (**B**) littoral habitat, outer inlet; (**C**) neritic habitat, inner inlet; and (**D**) neritic habitat, outer inlet. Early chum salmon fry were released on 10 and 17 May 2004 and 9–12 May 2005. Late fry were released 22–26 May 2004 and 22 May to 3 June 2005. Note difference in scale on *y*-axis.

habitat in the inner inlet in 2004 were caught in one set on 10 May.

As in littoral habitat, the greatest spatial and temporal overlap of chum salmon in neritic habitat occurred between wild and early hatchery fry in the outer inlet. Peak abundance of wild chum salmon in neritic habitat trailed behind the peak in littoral habitat by 1 to 2 weeks (Fig. 3,C and D). With one exception, no chum fry of any origin were caught in neritic habitat until the weeks of 17 May 2004 and 10 May 2005 (Fig. 3). Wild fry were most abundant in neritic habitat in both the inner and outer inlet during the week of 17 May

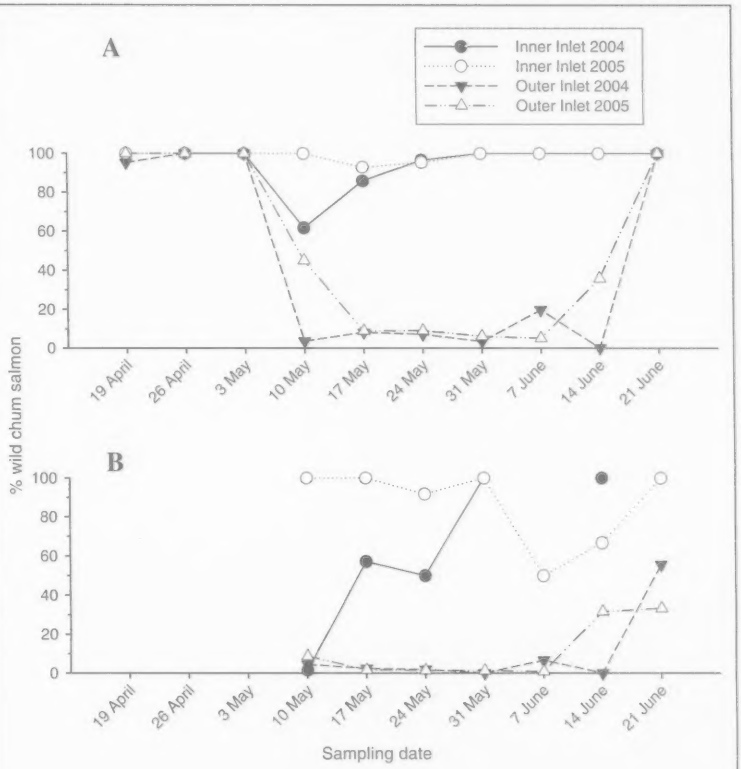

Figure 4

Weekly proportions of wild chum salmon (*Oncorhynchus keta*) fry from beach seine and Kodiak trawl sets in (**A**) littoral, and (**B**) neritic habitats of inner and outer Taku Inlet, Alaska, by week during the outmigration period for wild fry (late April to mid-June). No chum salmon were caught in neritic habitat before 10 May in 2004 and 2005. Early hatchery chum salmon were released near the inlet on 10 May and 17 May 2004 and 9–12 May 2005. Late hatchery fry were released 22–26 May 2004 and 22 May to 3 June 2005.

(Fig. 3). The proportion of wild fry was greatest in the inner inlet (Fig. 4), but abundance of both wild and hatchery chum salmon was greatest in the outer inlet.

Spatial and temporal change in body size of hatchery and wild salmon

Littoral habitat Early and late hatchery fry both were on average about four times heavier and about 15 mm FL longer than wild fry in littoral habitat (Fig. 5). Early hatchery fry were similar in size to late hatchery fry by the time the latter were released (Fig. 6). For wild fry in the inner inlet, mean weight and length were 0.39 g and 36.5 mm FL in 2004 and did not increase throughout the season (Fig. 6; regression probability that slope of size over time=0, P=0.62). In 2005, weight and length of wild fry did increase significantly (P=0.03), but the increases were relatively small, from 0.38 to 0.48 g and from 37 to 42 mm FL (Fig. 6). In the outer inlet, by contrast, wild fry more than doubled in weight and mean length increased significantly (P<0.01) through the season in both years, from 37 to 50 mm FL (Fig. 6). For early hatchery fry, mean length in the inner inlet increased significantly (P=0.02) over time from 51 to 54 mm FL in 2004, but did not change in 2005 (P=0.94). In the outer inlet, mean weight of early hatchery fry increased significantly (P<0.01) by 80% in both years and mean length increased significantly (P<0.01) from 52 to 60 mm FL in 2004 and from 53 to 63 mm FL in 2005 (Fig. 6). For late hatchery fry, mean length in littoral habitat in the outer inlet did not change significantly in either 2004 (P=0.24) or 2005 (P=0.08).

Neritic habitat Hatchery chum salmon fry in neritic habitat were longer than wild fish in the inner and outer

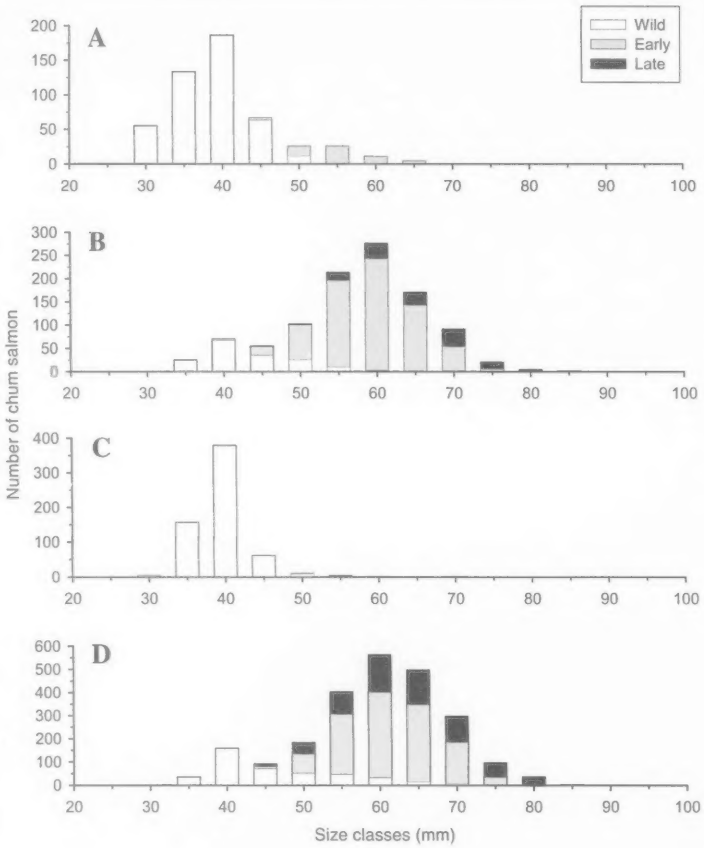

Figure 5

Size distribution (FL, mm) of wild chum salmon (*Oncorhynchus keta*) fry and early and late hatchery chum salmon fry caught by beach seine in littoral habitat in Taku Inlet, Alaska, during the outmigration period for wild fry (late April to mid-June): (**A**) inner inlet, 2004 (n=510); (**B**) outer inlet, 2004 (n=1,037); (**C**) inner inlet, 2005 (n=625); and (**D**) outer inlet, 2005 (n=2,379).

inlet in both years (Fig. 7). Late and early hatchery fry had similar fork length distributions in the outer inlet in both years. For wild fry, mean length in the inner inlet increased significantly over time in both years (Fig. 8), from 39 to 49 mm FL in 2004 (P=0.05) and from 36 to 47 mm FL in 2005 (P<0.01). In the outer inlet, mean length of wild fry increased significantly (P<0.01), from 42 to 66 mm FL in 2004 and from 41 to 74 mm FL in 2005. For early hatchery fry, mean length in the inner inlet increased significantly (P<0.01) from 54 to 62 mm FL in 2004, but sample size was too small for analysis in 2005. In the outer inlet, mean length of early hatchery fry increased significantly (P<0.01) in both years, from 53 to 69 mm FL in 2004 and from 59 to 67 mm FL in 2005. For late hatchery fry, mean length actually decreased significantly (P<0.01) in 2004 from 71 to 66 mm, but there was no significant change (P=0.56) in length in 2005 (Fig. 8).

Comparisons of habitats In both years, wild chum salmon fry in neritic habitat were larger in the outer inlet than in the inner inlet and larger in neritic habitat than in littoral habitat (Fig. 9; ANOVA: 2004: P<0.01, F=84.7; 2005: P<0.01, F=139.7). As with wild fry, early hatchery fry in 2004 were larger as they shifted from littoral habitat to neritic habitat (ANOVA: P<0.01, F=19.4); no significant differences were observed in 2005. Too few early hatchery fry were sampled in the neritic habitat in the inner inlet in 2005 to include in these analyses. In the outer inlet, fork length of late hatchery fry was significantly greater in neritic habitat than in littoral habitat in 2004 (t=1.97,

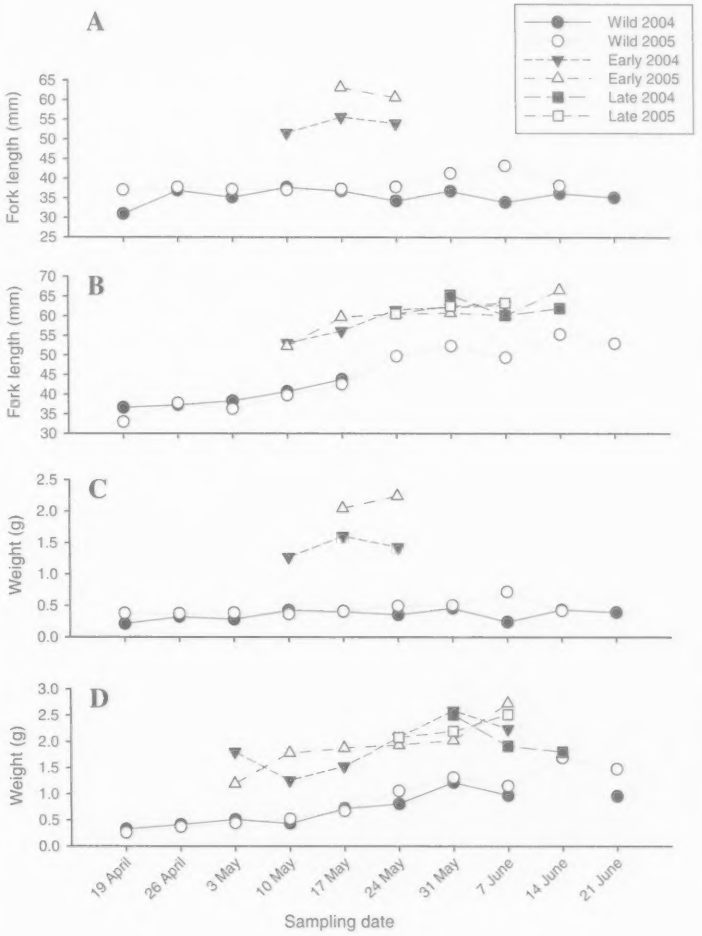

Figure 6

Size of wild and hatchery chum salmon (*Oncorhynchus keta*) fry caught by beach seine in littoral habitat of Taku Inlet, Alaska, by year during the outmigration period for wild fry (late April to mid-June): (**A**) fork length (mm), inner inlet; (**B**) fork length, outer inlet; (**C**) weight (g), inner inlet; and (**D**) weight, outer inlet. Early hatchery chum salmon were released near the inlet on 10 May and 17 May 2004 and 9–12 May 2005. Late hatchery fry were released 22–26 May 2004 and 22 May to 3 June 2005. No late hatchery fry were caught in the inner inlet in either year.

P=0.028), but no significant differences were found in 2005 (P=0.54).

Distribution, size, and condition of wild chum salmon fry in relation to temperature, salinity, and chum salmon abundance

Sea surface temperature and salinity followed patterns expected in Southeast Alaska in the spring during snowmelt (Meehan and Siniff, 1962; Murphy et al., 1997). Sea surface temperature generally increased throughout the season in littoral and neritic habitats in all locations and was warmer in the outer inlet than the inner inlet. Sea surface temperature ranged from 3° to 8°C in late April and increased to 7–12°C in late May and June in the inner inlet; in the outer inlet, it ranged from 4° to 9°C in late April and increased to 9–14°C in late May and June. Sea surface temperature

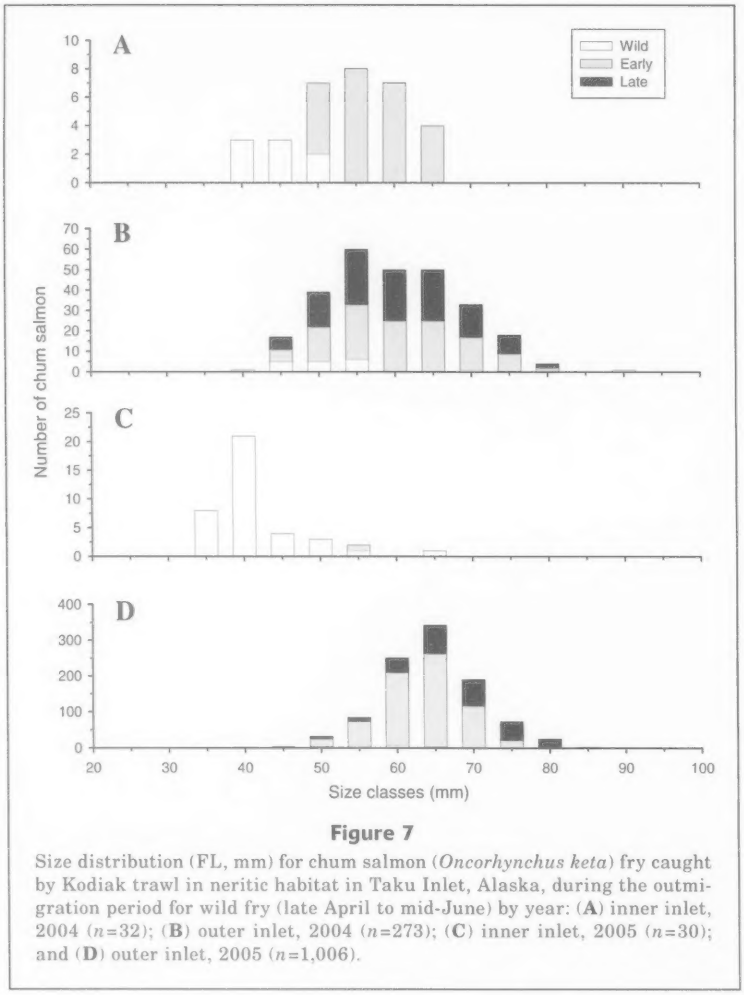

Figure 7

Size distribution (FL, mm) for chum salmon (*Oncorhynchus keta*) fry caught by Kodiak trawl in neritic habitat in Taku Inlet, Alaska, during the outmigration period for wild fry (late April to mid-June) by year: (**A**) inner inlet, 2004 (n=32); (**B**) outer inlet, 2004 (n=273); (**C**) inner inlet, 2005 (n=30); and (**D**) outer inlet, 2005 (n=1,006).

tended to be warmer in 2005 than in 2004, particularly after early hatchery fry were released. Salinity was also greater in the outer inlet than in the inner inlet in both littoral and neritic habitats. Salinity generally declined from 30 to 15 from April through mid-May in the outer inlet and remained relatively constant through the end of the season in both years, whereas in the inner inlet salinity declined from 15 to 5 over the same period.

Because of the small sample of wild chum salmon in neritic trawls, regression analysis of abundance, size, and condition of wild fish was limited to littoral habitats. In general, wild fry were more abundant and individuals were larger than hatchery fry. Abundance of wild fry was positively related to the abundance of hatchery fry in the inner inlet in 2004 and in the outer inlet in both years (Table 1). Abundance of wild fry was not related to salinity or sea surface temperature in either year.

Size (wet weight) of wild fry in the inner inlet was positively correlated with abundance of both wild and hatchery fry in 2004, but weight was negatively correlated with the abundance of wild fry and not correlated with the abundance of hatchery fry in 2005 (Table 2). Multiple regression analyses indicated that both wild and hatchery fry abundances were significant variables explaining the variation in weight in 2004; in 2005, neither parameter was significant when time (date) was included in the model (Table 2). Temperature was positively correlated with weight in both years but was not significant in the multiple regression models.

Weight of wild fry in the outer inlet was not correlated with either hatchery or wild fry abundance in 2004; only date was significant in the multiple regression analysis for the year (Table 2). In 2005, weight was negatively correlated with wild fry abundance and not correlated with hatchery fry abundance. However, when date was included in the multiple regression model,

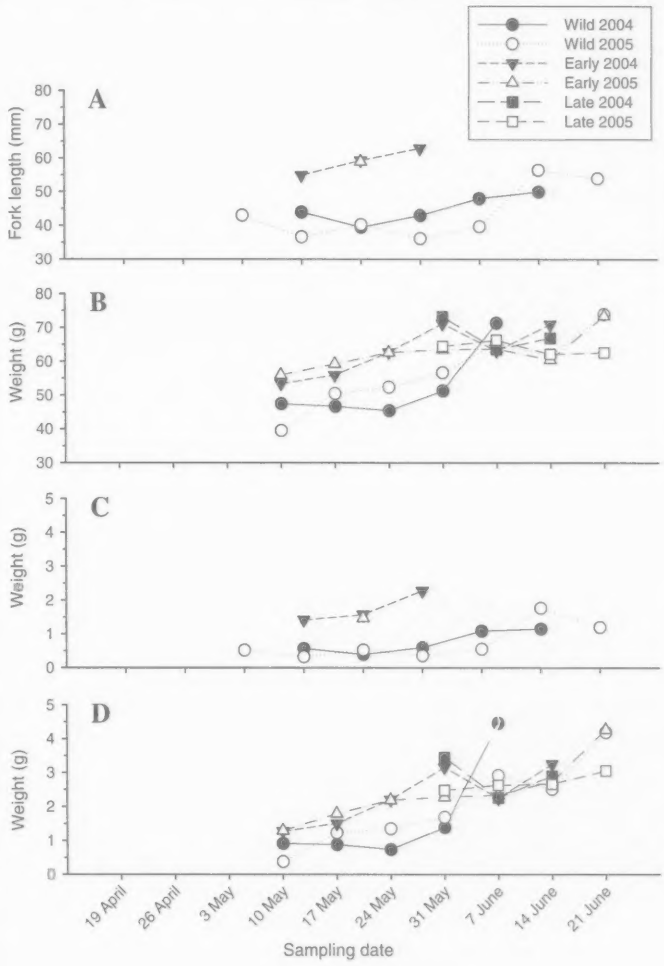

Figure 8

Fork length (FL, mm) and weight (g) of hatchery and wild chum salmon (*Oncorhynchus keta*) fry caught by Kodiak trawl in neritic habitats of Taku Inlet, Alaska, during the outmigration period for wild fry (late April to mid-June): (**A**) fork length (mm), inner inlet; (**B**) fork length, outer inlet; (**C**) weight (g) inner inlet; (**D**) weight, outer inlet. Early hatchery chum salmon were released near the inlet on 10 May and 17 May 2004 and 9–12 May 2005. Late hatchery fry were released 22–26 May 2004 and 22 May to 3 June 2005. No late hatchery fry were caught in the inner inlet in either year.

wild fry abundance had no significant effect on weight, whereas hatchery fry abundance had a significant positive effect on weight (Table 2). Sea surface temperature and salinity were positively and negatively correlated with weight, respectively, but neither variable was significant in the multiple regression analysis.

Condition factor of wild fry in the inner inlet was not correlated with either wild or hatchery fry abundance in 2004 (Table 3). In the multiple regression analysis, however, when salinity (which had the highest bivariate correlation) was included into the model, wild abundance had a significant and positive effect on condition factor. Hatchery abundance was not significant in the model. In 2005 in the inner inlet, condition factor was significantly correlated with wild fry abundance, but not with hatchery fry abundance (Table 3). In the

multiple regression analysis, wild abundance and date significantly affected condition factor in the inner inlet in 2005, and hatchery abundance had no significant effect.

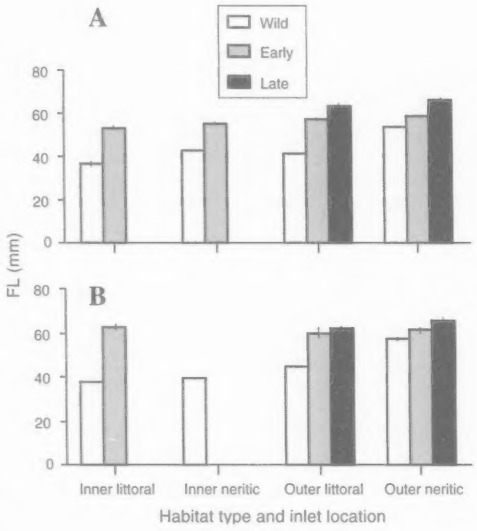

Figure 9

Fork length (mm) of chum salmon (*Oncorhynchus keta*) fry in Taku Inlet, Alaska, during the outmigration period for wild fry (late April to mid-June), by location and habitat in (**A**) 2004 and (**B**) 2005. Sample size for early hatchery fry in neritic habitat in the inner inlet in 2005 was too small to be included in analysis. No late hatchery fish were caught in the inner inlet during either year. Vertical bars represent standard error about the mean.

Condition factor of wild fry in the outer inlet was positively correlated with wild fry abundance but not with hatchery fry abundance in 2004 (Table 3). In the multiple regression analysis, wild fry abundance had a significant and positive effect on condition factor. Date also had a significant and positive effect, whereas salinity had a negative effect. Abundance of hatchery fry was not significant in the model in 2004. In 2005, condition factor of wild fry was not correlated with wild fry abundance but was positively correlated with hatchery fry abundance. In the multiple regression, hatchery fry abundance had a significant positive effect on condition factor, whereas wild fry abundance had a significant negative effect when included in the model with hatchery fry abundance (Table 3).

Discussion

The objectives for this study were 1) to determine the abundance and spatial and temporal overlap of wild chum salmon fry and hatchery chum salmon fry subjected to early and late rearing strategies in different habitats of Taku Inlet; 2) to compare the body size and condition of these groups of fry; and 3) to document environmental factors that potentially could explain the distribution, size, and abundance of fry. The ultimate goal for this study was to evaluate the potential for interactions between wild and hatchery chum salmon in Taku Inlet. Our results indicated that the greatest spatial and temporal overlap between wild and hatchery chum salmon fry occurred between wild and early hatchery fry in littoral and neritic habitats of the outer inlet. Both wild and early hatchery salmon were captured together in the same habitats in Taku Inlet for up to four weeks. Hatchery production corresponded with a 20-fold increase in overall abundance of chum salmon fry in the outer inlet, indicating a substantially increased likelihood of density-dependent interactions at this time (Simenstad et al., 1982; Levings et al., 1986; Willette,

Table 1

Relationships of wild chum salmon (*Oncorhynchus keta*) fry abundance to environmental factors and to hatchery chum salmon fry abundance from beach-seine collections in littoral habitat by inner and outer location in Taku Inlet, Alaska, during the outmigration period for wild fry (late April to mid-June), 2004–05, determined with stepwise multiple regressions (Eq. 1) and correlation analyses. SST = sea surface temperature; NS = not significant.

				Bivariate correlations with wild fry abundance			
		Significance of regression parameters		Inner		Outer	
		Inner	Outer	*r*	*P*	*r*	*P*
2004	hatchery abundance	Positive $P<0.01$	Positive $P<0.01$	0.365	<0.01	0.645	<0.01
	SST	NS	NS	0.198	0.110	0.027	0.865
	salinity	NS	NS	−0.246	0.047	0.099	0.526
2005	hatchery abundance	NS	Positive $P<0.01$	0.097	0.460	0.626	<0.01
	SST	NS	NS	−0.314	0.025	−0.200	0.205
	salinity	NS	NS	0.272	0.035	0.056	0.695

2001; Fukuwaka et al., 2007). Early hatchery fry were also notably larger than wild fry and larger fry are known to be favored in interactions among chum salmon fry (Olla et al., 1998). By comparison, the low overlap between the stocks in inner Taku Inlet is not surprising, given that hatchery chum salmon would have to migrate against a salinity-temperature gradient that they are adapted to follow seaward (Salo, 1991). The observation that apparent growth of wild fry was greatest in the outer inlet where hatchery fish were most abundant, and the lack of a negative relationship between condition of wild fry and hatchery fry abundance, would indicate that hatchery fry were not substantially depleting food resources available to wild fry and that negative, density-dependent interactions were not occurring or were not detected in this study. It should be noted, however, that apparent growth is potentially a biased measure of actual growth because of the continuous influx of small wild fry, shorter residence of larger fry, and size selective mortality, which have been documented for chum salmon

Table 2

Relationships of wild chum salmon (*Oncorhynchus keta*) fry weight to environmental factors and to hatchery chum salmon fry abundance from beach-seine collections in littoral habitat by inner and outer location in Taku Inlet, Alaska, during the out-migration period for wild fry (late April to mid-June), 2004–05, determined with stepwise multiple regressions (Eq. 2) and correlation analyses. SST = sea surface temperature; NS = not significant.

				Bivariate correlations with wild fry abundance			
		Significance of regression parameters		Inner		Outer	
		Inner	Outer	*r*	*P*	*r*	*P*
2004	date	NS	Positive P<0.01	−0.105	0.048	0.393	<0.01
	wild abundance	Positive P=0.051	NS	0.106	0.046	0.167	0.118
	hatchery abundance	Positive P=0.018	NS	0.127	0.017	0.005	0.961
	SST	NS	NS	0.096	0.071	0.219	0.039
	salinity	NS	NS	−0.012	0.825	−0.178	0.095
2005	date	Positive P<0.01	Positive P<0.01	0.350	<0.01	0.642	<0.01
	wild abundance	NS	NS	−0.129	0.034	−0.414	<0.01
	hatchery abundance	NS	Positive P<0.01	0.005	0.933	0.083	0.130
	SST	NS	NS	0.309	<0.01	0.576	<0.01
	salinity	NS	NS	−0.011	0.859	−0.318	<0.01

Table 3

Results of analyses relating wild chum salmon (*Oncorhynchus keta*) fry condition factor to environmental factors and to hatchery chum salmon fry abundance from beach-seine collections in littoral habitat by inner and outer location in Taku Inlet, Alaska, during the out-migration period for wild fry (late May to mid-June), 2004–05, determined with stepwise multiple regressions and correlation analyses (Eq. 3). SST = sea surface temperature; NS = not significant.

				Bivariate correlations with wild fry abundance			
		Significance of regression parameters		Inner		Outer	
		Inner	Outer	*r*	*P*	*r*	*P*
2004	date	NS	Positive P < 0.01	0.098	0.064	0.465	<0.01
	wild abundance	Positive P < 0.01	Positive P < 0.01	−0.036	0.503	0.481	<0.01
	hatchery abundance	NS	NS	−0.086	0.106	0.108	0.312
	SST	Negative P <0.01	Negative P = 0.015	−0.162	0.002	0.308	0.003
	salinity	NS	NS	−0.106	0.045	−0.164	0.124
2005	date	Positive P<0.01	NS	0.044	0.473	0.069	0.212
	wild abundance	Positive P<0.01	Negative P=0.045	0.254	<0.01	−0.088	0.110
	hatchery abundance	NS	Positive P<0.01	−0.079	0.198	0.278	<0.01
	SST	NS	NS	−0.060	0.396	0.162	0.101
	salinity	NS	NS	0.144	0.018	−0.166	0.234

fry during their early marine life history (Kaeriyama and Ueda, 1998; Wertheimer and Thrower, 2007).

To date, it is unclear how changes in fry abundance due to hatchery releases may affect predation risk and survival probability for wild chum salmon during their early life history. Changes in juvenile salmon abundance caused by hatchery releases can significantly change the dynamics of predator-prey interactions for wild fish (Einum and Fleming, 2001; Brannon et al., 2004). For example, the presence of hatchery fry could diminish predation on wild fry in the early marine phase by buffering wild fry from predators (Willette et al., 2001; Briscoe et al., 2005), could increase predation on wild fish by attracting predators and increasing predator-prey interactions (Holling, 1959; Beamish et al., 1992; Ruggerone and Rogers, 1984), or could lead to direct competition for food or space (Levings et al., 1986; Olla et al., 1998; Ruggerone and Nielsen, 2004). Size-selective mortality is not necessarily tied to hatchery practices or density-dependent interactions, but size-selective mortality would inflate our estimates of growth and condition. If such a bias did occur, it was probably not large enough to eliminate the apparent growth rates that we observed. Diminished growth and survival of wild fry may occur if the number of predators in relation to the number of salmon prey increases in response to increased hatchery releases (Ruggerone and Rogers, 1984; Beamish et al., 1992; Scheel and Hough, 1997), or if such an increase in fry abundance results in predators consuming wild chum salmon fry at a faster rate than they consume hatchery-produced fish (Holling, 1959).

The estuarine phase of the chum salmon life cycle in Taku Inlet provides ample time for interactions to occur that may influence survival of chum salmon fry, although this phase lasts less than a month. This short estuarine phase, however, is a critical period of rapid growth (Duffy et al., 2005; Simenstad et al., 1982; Wertheimer and Thrower, 2007), when fry must feed frequently to gain the energy to smolt, grow, avoid predators, migrate, and compete with other members of their cohort (Healey, 1982; Fukuwaka and Suzuki, 2002; Duffy and Beauchamp, 2008). In several studies on the daily mortality of chum salmon fry in estuaries, it was concluded that a large proportion of each cohort dies in the first 21 days at sea (Parker, 1962; Bax, 1983; Fukuwaka and Suzuki, 2002; Wertheimer and Thrower, 2007). Estuarine survivors often more than double in weight (Duffy et al., 2005) and larger fry are subsequently less susceptible to predation (Parker, 1971; Hargreaves and LeBrasseur, 1986; Wertheimer and Thrower, 2007). Similarly, survival of hatchery-reared chum salmon fry is influenced by body size at the time of release (Kaeriyama, 1999; Wertheimer and Thrower, 2007). Widespread conditions favorable to growth can increase survival of chum salmon cohorts from many stocks simultaneously (Pyper et al., 2002; Mueter et al., 2005; Duffy and Beauchamp, 2008), but interannual differences in environmental conditions are also reflected in size and survival rates (Wertheimer et al., 2004; Seo et al., 2006; Armstrong et al., 2008; Sturdevant et al., 2009).

New recruits to the inner inlet could create a negative bias in our estimates of apparent growth of wild fry because newly emigrated fry coming from the river would likely be smaller. In contrast, new recruits to the outer inlet come from the inner inlet and therefore the fact that wild fry were larger in the outer inlet than the inner inlet supports the conclusion that wild fry increased in fork length. Future research should include sampling near the mouth of the river itself for the benefit of comparing the size and outmigration timing of wild fry in the inner inlet with that of wild fry from the lower river. This bias did not exist for hatchery fry because there were no new recruits of hatchery fry after release. Although we do not have data on the length of time wild fry reside in the inner inlet, the fact that average length did not change substantially through the season indicates the catch could have been heavily influenced by new recruits. Our data indicated that at least some of the increase in length of wild fry in the outer inlet was due to actual growth. Fork length of early hatchery fry increased throughout the season in the outer inlet and wild fry also appeared to increase in size as they moved from the inner to the outer inlet. Early hatchery fry spent up to a month in the outer inlet and our catch data indicated that wild and early hatchery fry use habitat similarly. Consistent with other research (Healey, 1982; Mortensen et al., 2000; Duffy et al., 2005), both groups tended to be smaller in littoral than in neritic habitat, indicating that they exhibited the behavioral pattern of moving from shallow to deeper water with growth. Both size and predation risk can accelerate hatchery fry dispersal from nearshore habitats (Willette, 2001), which could also buffer the smaller wild fish from a different predator suite that coincides with this transition to offshore zones (Willette, 2001; Moss et al., 2005; Sturdevant et al., 2009).

Food availability for chum salmon fry may directly affect their survival, albeit to a lesser degree than predation risk (Mortensen et al., 2000; Willette, 2001; Willette et al., 2001). Based on a study of the bioenergetics of juvenile chum salmon in Icy Strait, Southeast Alaska, it was concluded that prey availability does not generally limit their growth (Orsi et al., 2004). However, compared to our early estuarine research, the study of Orsi et al. was conducted in epipelagic habitat and focused on larger hatchery and wild fish that had been in the marine environment for a minimum of 45 days; consequently, any competitive interactions may have occurred earlier. On the other hand, in studies of other estuaries of Southeast Alaska, spring carrying capacity far exceeded the estimated abundance of wild pink and chum salmon fry (Bailey et al., 1975), and fry rapidly outgrew predation vulnerability (Murphy et al., 1988). If estuarine conditions were equally favorable in Taku Inlet, hatchery fish may not directly compete with wild fish for food even when their densities are relatively high and the fish co-occur; instead, prey could be partitioned among size and stock groups of chum

salmon or according to foraging behavior and abilities, with little negative effect (Levings et al., 1986; Murphy et al., 1988; Sturdevant et al., 1996; Landingham et al., 1998). However, the timing of food resource availability in relation to estuarine entry of wild salmon or hatchery releases could affect residency time, diet, growth rate, predation, and survival (Hargreaves and LeBrasseur, 1986; Mortensen et al., 2000; Willette et al., 2001; Duffy and Beauchamp, 2008), and thus the extent and duration of potential interactions. The difference in environmental conditions that we observed in Taku Inlet in 2004 compared to 2005 indicates that this was a likely scenario. A companion study of diet and energy density of wild and hatchery chum salmon fry in Taku Inlet and Icy Strait is currently underway and should shed light on prey utilization, foraging behavior, and the extent to which hatchery and wild stocks partition food.

Interactions between hatchery and wild salmonids are complex and competition may occur only at critical periods during the life history of a cohort when resources are limited (Orsi et al., 2004; Ruggerone and Nielsen, 2004). Mixed schools of wild and hatchery fry formed in outer Taku Inlet, which may indicate that there is a potential for interactions as long as the schools persist. Both hatchery and wild juvenile chum salmon must learn to integrate many factors related to habitat, prey, and potential competitors and predators as they enter marine ecosystems (Willette et al., 2001; Warburton 2003; Armstrong et al., 2008; Duffy and Beauchamp, 2008). Hatchery salmon may rapidly learn to feed on natural prey after their release, yet these naïve fish also lack predator-recognition and -avoidance skills and may lag behind wild individuals in such abilities (Olla et al., 1998). Laboratory studies conducted with chum salmon indicate a potential for growth of wild fry to be affected by the presence of hatchery fry if there is a significant difference in body size, as we observed in Taku Inlet. This research indicates that larger individuals aggressively defend food when food is patchy but school with smaller fish when food is distributed evenly (Olla et al., 1998). On the other hand, we observed chum salmon fry in Taku Inlet before hatchery releases, and other studies concluded that despite smaller size, prior residence gives wild salmon a competitive advantage because the hatchery fish have to develop foraging behavior and search images for wild prey instead of hatchery pellets (Huntingford and Garcia de Leaniz, 1997; O'Connor et al., 2000). Later wild outmigrants in Taku Inlet also have the opportunity to develop foraging and predator-avoidance behavior in the inner inlet while few hatchery fish are present.

Although the focus of this article has been the potential for intraspecific interactions, the probability for interspecific interactions in Taku Inlet should not be overlooked, because these interspecific interactions may also occur in Taku Inlet. We captured considerable numbers of pink salmon fry that often co-occurred in similar habitats with chum salmon fry. Several studies have noted diet and habitat overlap between pink and chum salmon in their early marine life (Bailey et al., 1975; Sturdevant et al., 1996; Moulton, 1997; Duffy et al., 2005) or later (Landingham et al., 1998; Ruggerone and Nielsen, 2004). Commercial catches of pink salmon in Taku Inlet have been substantial, but variable, over the past 30 years and abundant populations of pink and chum salmon have co-existed in the Taku River. No data on historical abundance of pink salmon fry exist and there is no evidence that pink salmon returns have declined in the Taku River during the years since hatchery production of chum salmon began. The investigation of interspecific interactions, especially between pink and chum salmon, would be an important focus for future research.

Marine survival of most other chum salmon populations in Southeast Alaska has been stable (Orsi et al., 2004), and therefore poor ocean conditions are not the likely cause of the decline of wild chum salmon in the Taku River. Local evidence from the early ocean phase in epipelagic habitat has indicated that juvenile chum salmon consumed only a small portion of the available zooplankton (Orsi et al., 2004), and feeding indices have remained high throughout the diel cycle and summer season, indicating that growth of the fish was not food limited at this time. During the late ocean phase, run timing and harvest of adult wild and hatchery stocks are segregated in Taku Inlet; wild stocks return in the fall, whereas hatchery stocks (derived from broodstocks of summer-run chum salmon from coastal streams near Juneau) return in the summer. Although it is not known how many hatchery fish stray into the Taku River, this difference in run timing of adults presumably prevents large numbers of summer-run hatchery strays from interbreeding with the wild fall-run (Bachen and Linley, 1995; Heard et al., 1995). No directed fishery on wild Taku River chum salmon has operated since the early 1990s when the decline began. Wild fall-run chum salmon are intercepted in an annual coho salmon fishery in Taku Inlet and the catch of fall-run chum salmon in this fishery has averaged 4100 fish per year since 1992.

In summary, our results indicate that interactions in Taku Inlet between hatchery and wild chum salmon from the Taku River are possible because of the co-occurrence of these fish, particularly in the littoral habitat of the outer inlet, and the large proportion of early-released hatchery fry with larger body size. However, direct indications of competitive effects on wild fry, such as poor condition or reduced apparent growth rates in the presence of abundant hatchery fry, were not observed in this study. Because our understanding of the migration patterns of wild Taku chum salmon fry after leaving the inlet is inferred from data collected from hatchery fish, research to better define the degree of interaction should include a program to mark wild fry as they leave the river. Marking wild fry in the river would also allow a comparison of results of interactions such as growth, condition, feeding, and residence duration between wild and hatchery fry not only in the inlet but along their migratory corridor.

Our results also demonstrate that it is possible for hatcheries to successfully employ strategies that could reduce overlap between wild and hatchery fry and these strategies could apply to other salmonid hatcheries. DIPAC released fry near the outer inlet; because few hatchery fry entered the inner inlet, this release strategy reduced the potential for interactions with freshly emigrated small wild fish that are potentially more vulnerable. Potential negative interactions between early hatchery chum salmon and wild fish were also minimized by timing the release around periods of increased food resources and favorable temperatures for growth to reduce competition (Mortensen et al., 2000; Willette et al., 2001; Seo et al., 2006) and to minimize agonistic, size-related behavior (Olla et al. 1998); later releases of chum salmon fry may provide the best chance for the fish to avoid predation (Olla et al., 1998; Hawkins et al., 2008), and our data demonstrate that these later release fry will likely emigrate to sea more quickly, a strategy that could be useful to fishery managers seeking to reduce the potential for interactions between wild and hatchery fry during the critical life stage of estuarine and early marine residence.

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Prediction of discard mortality for Alaskan crabs after exposure to freezing temperatures, based on a reflex impairment index

Allan W. Stoner

Fisheries Behavioral Ecology Program
Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
2030 S. Marine Science Drive
Newport, Oregon 97365

Email address for author: al.stoner@noaa.gov

Abstract—Millions of crabs are sorted and discarded in freezing conditions each year in Alaskan fisheries for Tanner crab (*Chionoecetes bairdi*) and snow crab (*C. opilio*). However, cold exposures vary widely over the fishing season and among different vessels, and mortalities are difficult to estimate. A shipboard experiment was conducted to determine whether simple behavioral observations can be used to evaluate crab condition after low-temperature exposures. Crabs were systematically subjected to cold in seven different exposure treatments. They were then tested for righting behavior and six different reflex actions and held to monitor mortality. Crabs lost limbs, showed reflex impairment, and died in direct proportion to increases in cold exposure. Righting behavior was a poor predictor of mortality, whereas reflex impairment (scored as the sum of reflex actions that were lost) was an excellent predictor. This composite index could be measured quickly and easily in hand, and logistic regression revealed that the relationship between reflex impairment and mortality correctly predicted 80.0% of the mortality and survival for *C. bairdi*, and 79.4% for *C. opilio*. These relationships provide substantial improvements over earlier approaches to mortality estimation and were independent of crab size and exposure temperature.

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Fishes and invertebrates are discarded from fishing operations in ever increasing quantities (Alverson et al., 1994; Cook, 2003; Broadhurst et al., 2006), and the various components of bycatch-related mortality, including detected, undetected, immediate, and delayed forms of mortality have been discussed in recent reviews (Hall et al., 2000; Davis, 2002). Crabs, shrimps, and lobsters are discarded in high proportions in relation to landings in both the directed fisheries for crustaceans and in the prosecution of finfish fishing (Cook, 2003), and many of those discards die from stressors including physical injuries to the carapace, lost and broken limbs, and physiological stress associated with temperature changes and air exposure.

Davis (2002) reviewed general principles of bycatch-related stressors and concluded that some aspects of handling and discard mortality can be simulated in the laboratory. This simulation has been undertaken extensively for fishes in recent years (see Davis, 2002), and controlled laboratory exposures to stressors relevant to fishing, such as air exposure and dropping of crabs (during handling on ships), have been conducted for lobsters (Brown and Caputi, 1983; DiNardo et al., 2002; Harris and Ulmestrand, 2004) and crabs (Zhou and Shirley, 1995; Grant 2003). Field studies designed to test different handling methods for discards typically employ either a tag and recovery approach (Brown and Caputi, 1983; Watson and Pengilly, 1994) or some means of holding the test animals in field enclosures (Kennelly et al., 1990; Grant, 2003; Broadhurst et al., 2009) or tanks (DiNardo et al., 2002; Stoner et al., 2008). Although direct experimental observations on mortality are useful, tag studies often yield relatively low returns, and experiments requiring holding can ordinarily accommodate only a relatively low number of treatment types and limited replication.

As an alternative to observing mortality directly it is sometimes possible to apply a measure of animal condition that is closely associated with delayed mortality. For example, Shirley and Stickle (1982) suggested that righting behavior (i.e., an animal's ability to turn from a ventrum-up position to normal orientation) is a complex reflex requiring muscle coordination and neurological control that can be a sensitive measure of well-being. Righting behavior has been observed in several studies with Alaskan crabs (Stevens, 1990; Carls and O'Clair, 1995; Zhou and Shirley, 1995; Warrenchuk and Shirley, 2002), and others have scored vitality of crabs on the basis of spontaneous movements of the appendages in order to predict delayed mortality (Stevens, 1990; Purves et al., 2003). More recently, Stoner et al. (2008) developed an extension of the vitality metric, exploring a suite of six reflex actions (Table 1) that reflect the condition of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) injured in bottom trawl operations. These reflexes are stereotypic and can be evaluated rapidly in the tester's hand (out of water) during shipboard operations,

Table 1

Reflexes identified as useful for assessing stress in *Chionoecetes* spp. "Test" is the manipulation required to elicit a stereotypic positive response. No response was recorded when no motion was detected in response to repeated testing (modified from Stoner et al., 2008)

Reflex	Test	Positive response	Lost response
Leg flare	Lift crab by the carapace, dorsum up	Legs spread wide and to near horizontal orientation in strong crabs	Legs droop below horizontal, with no attempt to raise them
Leg retraction	While holding crab as above, draw the forward-most walking legs in the anterior direction	Legs retract in the posterior direction, or present resistance to the motion in weakened crabs	No resistance to the manipulation occurs
Chela closure	Observe for motion or hold the chelae in the fingers	Chelae open and close with or without manipulation. In weakened crabs the chelae may close slowly, or show low resistance to manual opening	No motion is detected in the chelae under manipulation
Eye retraction	Touch the eye stalk with a blunt probe, or lift the eye stalk from its retracted position	Eye stalk retracts in the lateral direction below the carapace hood, or shows resistance to lifting	No motion or resistance to manipulation occurs in the eye stalk
Mouth closure	If closed, attempt to open (extend) the 3rd maxillipeds with a sharp dissecting probe. If open, draw the maxillipeds downward	3rd maxillipeds retract to cover the smaller mouth parts. The maxillipeds droop open or move in an agitated manner in weakened crabs	No motion in the maxillipeds occurs
Kick	With the crab in ventrum-up position, use a sharp dissecting probe to lift the abdominal flap away from the body	One or more legs or chelipeds move quickly in the ventral direction, particularly in males. Motion in the hind most legs is retained in weakened crabs	No motion in the legs or chelipeds occurs

with a high degree of reliability. More importantly, a composite index of reflex impairment (i.e., the simple sum of reflex actions lost) provided a graded metric for the animal's condition [0 to 6], and was a good predictor for mortality in field trials. Relationships between reflex impairment and mortality were first explored for fishes (Davis, 2002, 2007) and the results were termed a reflex action mortality predictor (RAMP) by Davis and Ottmar (2006). These recent experiments have shown that RAMP models, once established for a species, provide reliable predictors independent of crab size, molting stage, gender, and injury. The RAMP approach has the added benefit of eliminating the need for holding the animals tested.

The subject species of this study were *C. bairdi* and *C. opilio*. Landings of *C. bairdi* reached maximum values (>100 million pounds) in Alaska during the late 1970s but have been relatively low since a fisheries collapse in the mid-1980s (Herrmann and Greenberg, 2007). *Chionoecetes opilio* is distributed more widely, from the Sea of Japan to Alaska in the North Pacific and through the Arctic Ocean to Atlantic Canada. During the early 1990s landings for *C. opilio* exceeded 300 million pounds in Alaska and over 100 million pounds in the North Atlantic (Herrmann and Greenberg, 2007). Peak value in Alaska occurred in 1994 at $200 million, then declined sharply, with Canadian fisheries in the Atlantic now representing >80% of the catch.

Fishing for *Chionoecetes* spp. in Alaska is limited to baited pots and only males of minimum size may be retained. Consequently, all crabs captured in trawl fishing are returned to the sea, and large numbers of sub-legal-size males and all female crabs caught in pots must be returned to the water. Many males exceeding minimum size are also returned. MacIntosh et al. (1996) discussed the magnitude of discards in the Bering Sea fishery, noting that discards could represent a large source of population mortality if these incidentally captured crabs die. Because directed fisheries for *C. bairdi* and *C. opilio* occur primarily during winter months in Alaska, the crabs can experience periods of freezing temperature and wind chill before being discarded. For calculations of catch limits for US-managed crab fisheries in the Bering Sea, both the retained commercial catch and the numbers of discarded crabs which die due to handling mortality are considered to be part

of the total catch (NPFMC, 2008). Currently, a rate of 50% mortality is applied to the crab discarded from pot fisheries in Alaska (Turnock and Rugulo, 2008), but it is widely recognized that more research is needed to refine this value and to improve handling methods to reduce discard mortality.

The goal of this study was to determine if the RAMP approach can be applied to thermal stress experienced by *C. bairdi* and *C. opilio* in Alaska fisheries prosecuted during winter months. In a shipboard experiment, males of both species at submarket size were subjected to different subfreezing temperatures and exposure times to test for possible relationships between simple reflex actions, righting behavior, autotomy, and subsequent immediate mortality and delayed mortality.

Materials and methods

Field collections and experimental animals

Crabs for this study were collected with the 50-m fishing vessel *Pacific Explorer* in waters east of St Paul Island (Pribilof Islands), in the Bering Sea near 57°03′N, 168°20′W, at 80 m depth. During the August 2008 field work, bottom water temperature was 1.1° to 1.5°C. Crabs were collected in various locations around a commercial trawl with a recapture net previously described for studies of crab bycatch injury (Rose, 1999; Stoner et al., 2008). The main trawl was a two-seam Alfredo bottom trawl (with headrope and footrope lengths of 36 m and 54.6 m, respectively) similar to that used by many vessels in the Bering Sea. This trawl was towed with an open codend. The recapture net was a small 2-seam trawl with a longer headrope than footrope (14.3 m and 12.0 m, respectively). The long headrope maximized escape of fish, and a small-diameter (5 cm) footrope was used to enhance crab capture. For the broader role of the research cruise, evaluating crab injury caused by various gear components, crabs were collected at various locations around the primary net (e.g., behind the main net sweeps, wings, or footrope). As a control for damage in the recapture nets, crabs were also fished directly ahead of the main trawl, so that they were captured with no previous damage. Crabs from this net position were used for the study of freeze-related injury reported here. Tows were short (15 minutes) so that crab stress was minimized.

Once a recapture net was on deck, *C. bairdi* and *C. opilio* were sorted quickly from the catch and tested individually (in air) for losses of six previously studied reflexes (Table 1) (Stoner et al., 2008). For this study, experiments were limited to crabs meeting the following criteria: 1) males below market size (i.e., *C. bairdi* = 80–100 mm carapace width [CW]; *C. opilio* = 71–100 mm CW); 2) crabs without apparent physical injuries or limb loss; 3) crabs with carapaces in full hardness; and 4) crabs in perfect condition as revealed by reflex actions. Crabs in the above prescribed size classes represent the primary discards observed in Alaska pot fisheries (see Warrenchuk and Shirley, 2002). Air temperature during the crab handling process ranged from 5° to 10°C, and sorting normally took <15 minutes. Crabs meeting the criteria specified were immediately moved to one of 12 large fish totes (98×110×85 cm deep; ~900 liters) secured on the trawl deck. Each tote was supplied with a constant flow of seawater (≥20 L/min). These systems were identical to those used in an earlier study of crab mortality (Stoner et al., 2008). Water temperature during the pre- and post-testing holding period ranged from 9.1° to 9.6°C, salinity was nearly constant at 27.2 psu, and oxygen (monitored morning and evening in every tote) never fell below 100% saturation. The holding temperature was higher than desired, but no control crab (see below) died or showed signs of stress in the form of impaired reflexes.

Experimental systems and rationale for temperature exposures

Crabs were exposed to freezing temperatures in a standard chest freezer (internal dimensions 76×46×71 cm deep) secured below deck on the FV *Pacific Explorer*. Temperature was monitored with a digital instrument and cabled resistance temperature detector (RTD) platinum probe (0.1°C resolution, Fisher Scientific, Pittsburgh, PA) fastened at the bottom of the freezer compartment near the subject crabs. This digital thermometer was calibrated against a standard mercury thermometer. A 5-cm-thick layer of ice in the bottom of the freezer helped to stabilize the air temperature on the bottom of the compartment where crabs were placed ~3 mm off the bottom surface in an open mesh plastic rack. Preliminary experimentation with a small fan inside the freezer compartment showed that temperature stability at the bottom of the freezer was greatest without air circulation, and this eliminated the complicating factor of wind chill. Although crabs in the fishing conditions would rarely be exposed to freezing temperatures without at least some wind, the primary objective of this study was to determine how well reflex actions would reflect thermal stress in crabs and predict mortality, not to provide absolute values of mortality under specific outdoor conditions.

The strategy for thermal exposures was guided by the results of earlier cold-exposure experiments conducted by Carls and O'Clair (1990, 1995). They found that responses to cold air in *C. bairdi* and *Paralithodes camtschaticus* were best described when the units of exposure were considered as the product of temperature and duration of exposure (h), over a range of temperatures from –20° to 5°C. For example, short exposures at low temperature caused the same effects as long exposures at a higher temperature when the units of exposure (degree hours,°h) were equal. The same units of measurement were used in this study.

The objective of this investigation was to determine whether stress (and mortality) caused by cold exposure can be predicted from a reflex impairment index; therefore, it was important to generate variable levels

Table 2

Summary of exposures of crabs to freezing temperatures: *Chionoecetes bairdi* (Tanner crab) (80–100 mm carapace width [CW]) and *C. opilio* (snow crab) (71–100 mm CW). Treatments are expressed in degree-hours (°h)—the product of temperature (in degrees Celsius) and time (in hours). Values for actual temperature and time of exposure are reported as means and standard deviations. Ten crabs were tested in each treatment, except for the –8°h exposure for *C. opilio* at –20°C where 12 crabs were tested.

		C. bairdi		*C. opilio*	
Nominal temperature (°C)	Exposure treatment (°h)	Actual temperature (°C)	Exposure time (min)	Actual temperature (°C)	Exposure time (min)
–20	–2	–17.6 ±3.1	7.3 ±1.8	–17.2 ±3.7	7.3 ±1.8
	–3	–17.6 ±2.2	10.5 ±1.5	–17.0 ±2.9	10.5 ±1.9
	–4	–17.0 ±2.8	14.5 ±2.8	–16.6 ±2.9	14.8 ±2.4
	–5	–19.0 ±1.6	15.8 ±1.2	–17.8 ±3.1	17.2 ±3.4
	–6	–18.2 ±1.8	20.0 ±2.2	–16.6 ±2.3	22.2 ±3.3
	–8	–19.6 ±0.5	24.2 ±0.5	–17.2 ±2.4	28.6 ±4.3
	–10	–16.3 ±3.5	40.8 ±9.3	–17.0 ±2.5	36.6 ±4.4
–10	–2	–10.0 ±0.7	12.0 ±0.7	–10.2 ±0.5	12.0 ±1.6
	–6	–11.0 ±0.7	33.0 ±2.0	–10.6 ±0.5	33.6 ±1.2
	–8	–10.4 ±0.5	46.4 ±2.1	–10.4 ±0.5	46.4 ±2.0

of cold-related stress that would result in a full range of impairments (0 to 6; see below). Mortality in *C. bairdi* observed by Carls and O'Clair (1990) increased in a clear sigmoid pattern from 0% to 100% with increasingly severe exposures from approximately –2 to –7°h. Therefore, exposures of –2, –3, –4, –5, –6, –8 and –10°h were chosen for this study. These exposures were achieved with two nominal temperatures, –20°C for primary experiments, and additional tests at –10°C to test for the generality of the degree-hour approach (Table 2). Temperature excursions of 1–3°C sometimes occurred during a run, particularly when the largest crabs were placed in the small freezer, but the degree-hour exposure desired could be easily achieved by systematically increasing or decreasing exposure time for an individual run.

Experimental protocol

Crabs for this study were held for at least 48 h before initiating experiments on cold stress. Each crab was inspected for injury and retested for reflexes just before experimentation to insure that only crabs in perfect condition were used. In fact, few crabs in diminished condition were found among those initially selected for holding (<1%), indicating that the holding environment was suitable.

Experiments were typically conducted with pairs of crabs. This procedure ensured that exposures to freezing temperatures and subsequent handling for each crab could follow a strictly timed protocol, and that uniform postexposure testing was possible. First, the test crabs were marked with uniquely numbered vinyl spaghetti tags tied securely but loosely around the basi-ischium of the third or fourth leg. The crabs were then quickly placed on the freezer rack (at a preset temperature), ventrum up to prevent excessive movement. Temperature of the freezer was closely monitored during the exposure, and exposure times were adjusted to maintain the prescribed degree-hour treatment. The seven different exposures were interspersed over the course of runs made at each experimental temperature (–20° and –10°C) until 10 or 12 replicates of each exposure were completed (Table 2).

After exposure to the cold each crab was evaluated with a series of three basic tests of behavioral capabilities. 1) Immediately following removal from the freezer the crab was tested for the presence or absence of the six reflex actions (Table 1). 2) Then, the crab was laid ventrum up in a flowing seawater bath (dimensions= 80×50×30 cm deep; 8–9°C) and observed for 120 seconds. Attempts by the crab to turn dorsum up were recorded, and if it was successful the time to right was recorded. This procedure was identical to that used by Carls and O'Clair (1990). Although crabs placed in this position in the freezer normally lay quietly, the natural tendency of crabs in water is to quickly turn dorsum up. 3) After precisely 120 sec in the water bath, the crab was removed from the water and reflex actions were retested. After these tests, any autotomies were recorded (identifying the specific limbs missing), and the crab was returned to a large recovery tank for monitoring of mortality. Mortality was assessed and dead crabs were removed each day until the end of the experiment when reflexes were re-assessed for all of the living crabs. Autotomies were recorded for dead crabs and for all live crabs at the end of the experiment. Tests for the two species spanned six days, and each individual was

monitored for nine days. This monitoring period was determined by the duration of the vessel charter, and justified by Carl and O'Clair's (1995) observation that "almost all mortality occurred 1–2 days after exposure to freezing temperatures."

To ensure that holding conditions did not affect the condition of crabs during posttreatment monitoring, additional crabs collected in the trawls were set aside for routine handling and monitoring in the same manner as that for the test subjects. Males (n=20 *C. bairdi* and n=34 *C. opilio*) with perfect reflex scores and in size ranges equivalent to the experimental crabs were removed from the holding tanks daily and monitored for mortality. None of these crabs died and all had perfect reflex scores at the end of the experimental period, indicating that the holding conditions were adequate despite water temperature higher than where the crabs were collected.

Reflex impairment indices and statistical procedures

Scores for reflex actions were combined into a composite impairment index. This index, simply the sum of reflex actions lost, ranges from 0 to 6. Composites provide robust indices of overall condition for the animal and have the advantage of reducing the weight of any one reflex (Davis, 2007). Analyses described below were conducted with two different impairment indices, one calculated for reflexes assessed immediately after removal from the freezer (index A) and the other after two minutes in a water bath (index B)(see above).

As with the statistical approach of Stoner et al. (2008), a logistic regression was used to model mortality with potential predictors and mediators, namely reflex impairment, experimental temperature, and crab size. Models were fitted by the method of maximum likelihood for binary data (i.e., dead or alive) by using the regression module of Systat 12 (SYSTAT Software, Inc., San Jose, CA) (Peduzzi et al., 1980). A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model. This model for mortality was described by the following equation:

$$Log_e\left(p/(1-p)\right)=\alpha+\beta' x, \quad (1)$$

where p = proportion of y=1;
y = 1 if dead and 0 if alive;
α = intercept;
β' = model coefficients; and
x = the model matrix of explanatory variables.

The maximum likelihood estimates of mortality (ρ) were calculated as

$$\rho=e^{(\alpha+\beta' x)}/1+e^{(\alpha+\beta' x)}. \quad (2)$$

Initially, the data for each species were split randomly into equal halves, one representing a learning set and the other a test set. The most parsimonious logistic model was developed by using the learning set that was then validated with the test set. After cross-validation, a final model was fitted to the entire data set. Finally, the logistic model for each species was used to develop a response curve showing the probability of mortality based upon fixed values for the key observations of crab condition.

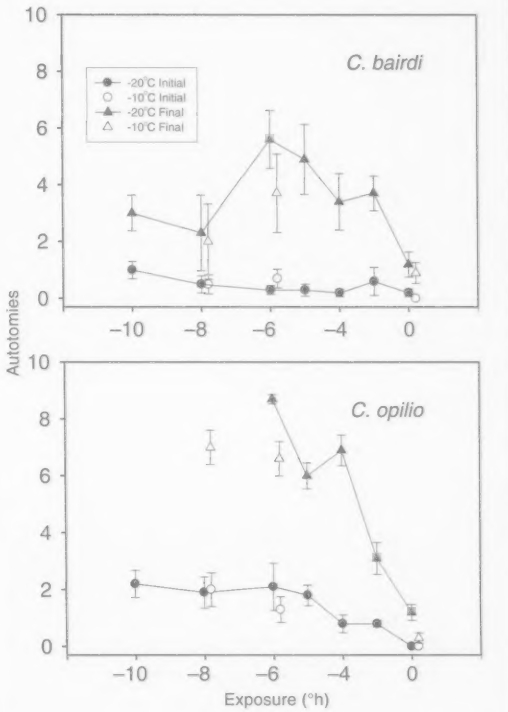

Figure 1

Numbers of limbs autotomized by *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) during and immediately after freezing (circles) and at the end of a 9-day holding period (triangles). Exposure treatments were made at two temperatures and are plotted in units of degree-hours (°h, the product of temperature [in degrees Celsius] and time [in hours]).

Results

Autotomy

Autotomy during and immediately after freezing conditions increased in direct proportion to cold exposure and was generally higher in *C. opilio* than *C. bairdi* (Fig. 1). *Chionoecetes opilio* lost two legs on average in exposures ranging from –4 to –10°h, and none at –2°h. Average losses in *C. bairdi* were less than one across the

Table 3

Righting behavior exhibited by crabs exposed to different levels of freezing exposure measured in degree-hours (°h, the product of temperature [in degrees Celsius] and time [in hours]). Values in parentheses represent the proportions of crabs attempting to right and righting successfully that survived. 60.9% of surviving *Chionoecetes bairdi* (Tanner crab) and 52.6% of surviving *C. opilio* (snow crab) made no attempt to right.

Exposure level (°h)	No. of crabs tested	No. of crabs attempting to right (% survival)	No. of crabs righting successfully (% survival)	Range of righting time (seconds)
C. opilio				
–2	20	10 (90)	7 (100)	19–92
–3	10	4 (100)	0	—
–4	10	3 (67)	2 (100)	90–98
–5	10	4 (50)	2 (50)	80–88
–6	20	8 (25)	0	—
–8	22	3 (33)	0	—
–10	10	0	0	—
Overall	100	32 (62.5)	11 (90.9)	19–98
C. bairdi				
–2	20	13 (92)	9 (89)	5–110
–3	10	2 (50)	1 (100)	12
–4	10	1 (100)	0	—
–5	10	1 (100)	0	—
–6	20	5 (40)	0	—
–8	20	1 (0)	0	—
–10	10	0	0	—
Overall	100	23 (73.9)	10 (80.0)	5–110

exposure range. When two different temperatures (–10° and –20°C) were used to create fixed exposure levels (°h) differences in initial autotomy were not different for either species (ANOVA, $P>0.60$). In *C. opilio*, the chelipeds were infrequently lost (4.8%) and limb losses increased in the anterior to posterior direction (Fig. 2). Losses were more uniformly distributed in *C. bairdi*. Although direct comparisons with the initial limb losses were not possible because of high mortalities in some treatments, autotomies increased substantially over the 9-day holding period, especially in crabs exposed to freezing temperatures for longer periods (Fig. 1).

Righting behavior

Crabs of both species attempted to right themselves when placed in the water bath after exposure to freezing temperatures (Table 3), but the total number of successful crabs was relatively low (10–11%). Most of the crabs able to right themselves survived over the 9-day follow-up period (90.9% of *C. opilio*, 80% of *C. bairdi*). However, neither attempts to right nor time to right were good predictors of subsequent survival because 52.6% of surviving *C. opilio* and 60.9% of surviving *C. bairdi* made no attempt to right. Also, crabs demonstrating fast righting times (e.g., 12 sec) sometimes died. Overall, successful righting was a relatively good predictor of survival, but neither the lack of righting nor attempts to right were good predictors of mortality.

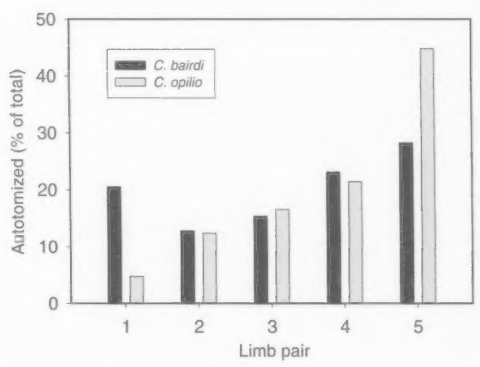

Figure 2

Identities of limbs autotomized by *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) after exposure to freezing conditions (all treatments combined). Limb pairs were numbered 1–5, from the chelipeds to the posteriormost walking legs.

Cold exposure, mortality, and reflex impairment

Crab mortality increased substantially with increasing exposure to freezing stress (Fig. 3), as expected. With exposures ranging from –2 to –4°h , mortalities

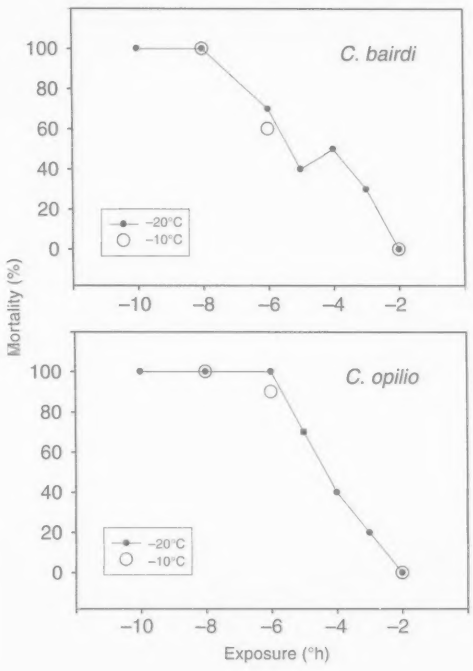

Figure 3

Percent mortality of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) shown as a function of exposure to freezing conditions. Exposure treatments were made at two temperatures and are plotted in units of degree-hours (°h, the product of temperature [in degrees Celsius] and time [in hours]).

were relatively similar in *C. bairdi* and *C. opilio*, then rose more quickly in *C. opilio*, reaching 100% at an exposure of −6°h. The results for crabs exposed at −10° and −20°C were nearly identical. Large proportions of the total mortality values for both *C. bairdi* and *C. opilio* occurred within 24 hours and then continued in a near linear pattern until the end of the 9-day holding period (Fig. 4). First-day mortality was especially high in *C. opilio*, accounting for more than 60% of the total mortalities observed. Eighty percent of mortality was observed by day 5 and day 6 for *C. opilio* and *C. bairdi*, respectively.

Exposure to freezing temperatures resulted in substantial impairments of reflex actions, both immediately after removal from the freezer (index A) and after the 2-minute warming period in a water bath (index B). However, preliminary analyses showed that index B had two critical limitations in terms of association with subsequent crab mortality. First, 32–35% of crabs with apparently perfect reflex scores (index B=0) died over the following 9 days. Second, mortality in *C. opilio* reached 100% at all impairment scores greater than 1.

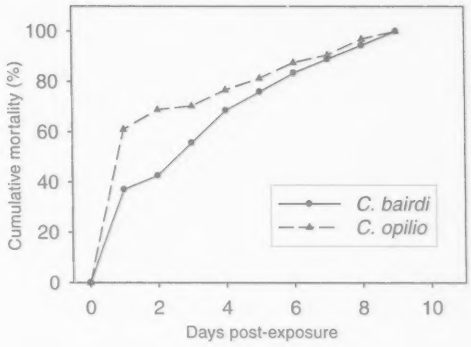

Figure 4

Cumulative percentage of total mortality for *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) over time after exposure to freezing conditions. Total numbers of *C. bairdi* and *C. opilio* dying over the course of the experiment were 54 (54% of 100 tested) and 62 (60.8% of 102 tested), respectively.

These flaws make index B a weak predictor of mortality, and this impairment index was considered no further. The results from index A were far superior. Mortality increased smoothly from an impairment of 0 to 6 in both species, and relatively little mortality occurred with an index value of 0. All of the following results are reported with respect to index A.

Reflex impairment score increased in a near linear pattern with increasing exposure to freezing temperatures (Fig. 5). For both *C. bairdi* and *C. opilio*, mean impairments were just above 0 with an exposure of −2°h and increased steadily to 5.5 and 6.0 (loss of all reflexes tested) at −10°h. The variation was remarkably small for *C. opilio*, although crabs in the two longest exposures conducted at −10°C (−6 and −8°h) fell well below the curve for trials conducted at −20°C. The effect of the test temperature on impairment was significant for *C. opilio* (ANOVA, $P<0.001$), but not for *C. bairdi* ($P=0.435$).

Patterns occurred with reflex impairments. The most sensitive reflex, lost first in 40% or more cases, was the ability to close chelae (Table 4). Kick and eye retraction were also lost first in substantial numbers of crabs. Reflex action of the mouth was least likely to be the first reflex lost, and among all of the losses observed this response was lost in just 0.4% (*C. bairdi*) and 1.0% (*C. opilio*) of individuals with impairments scoring 1 to 5. All other reflexes were lost in substantial numbers for both species (Table 4).

Reflex impairment (index A) was a good predictor of mortality for *C. bairdi* and *C. opilio* and was independent from test temperature and crab size (logistic regression). The most parsimonious model for *C. bairdi*, containing only a constant and reflex impairment, correctly predicted 80.0% of the mortality and survival (Table 5), whereas the full model (with all variables)

Table 4

Percentages of reflex actions lost in *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab). When only one reflex was absent in a crab it was considered the 1st reflex lost. In all cases where only one reflex remained that reflex was mouth closure. The right column represents the percentage of specific reflexes that were lost among all of the crabs where between one and five reflexes were lost.

Reflex	1st reflex lost	% of total losses
C. bairdi	(*n*=20)	(*n*=227)
Chela closure	40.0	25.6
Kick	35.0	24.7
Eye retraction	20.0	18.9
Leg flare	5.0	14.1
Leg retraction	0	16.3
Mouth closure	0	0.4
C. opilio	(*n*=14)	(*n*=201)
Chela closure	42.9	27.9
Kick	14.3	20.9
Eye retraction	21.4	16.4
Leg flare	14.3	16.9
Leg retraction	7.1	16.9
Mouth closure	0	1.0

Table 5

Results of logistic modeling for mortality in *Chionoecetes bairdi* (Tanner crab). A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model.

Parameter	Estimate	*Z*	*P* value
Full model			
constant	–7.466	–1.789	0.074
temperature	0.008	0.013	0.990
crab size	0.058	1.321	0.187
reflex impairment	0.877	5.900	<0.001
Most parsimonious model			
constant	–1.953	–4.427	<0.001
reflex impairment	0.833	5.568	<0.001

Prediction matrix for the most parsimonious model

	Dead predicted	Live predicted	Actual total
Die	42	11	53
Live	9	38	47
Total no. predicted	51	49	100
Correct (%)	79.2	80.9	
False (%)	26.2	33.9	
Total correct (%)			80.0

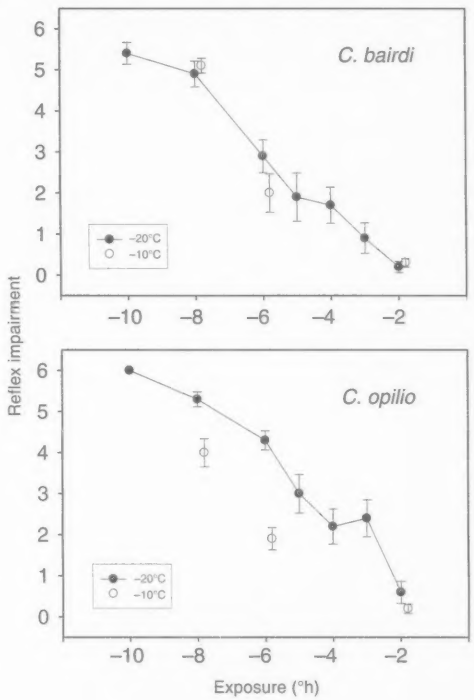

Figure 5

Reflex impairment observed in *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) immediately after exposure to seven treatments at different freezing conditions. Exposure treatments were made at two temperatures and are plotted in units of degree-hours (°h, the product of temperature [in degrees Celsius] and time [in hours]).

predicted 81.0% correctly. The model for a random learning set (one half of the data) incorporated the same statistically significant variables (constant and reflex impairment) and correctly predicted 75.5% of the results for an independent test set. A similar analysis for *C. opilio* resulted in a full model that predicted 80.4% of mortality and survival correctly, with only reflex impairment having a significant effect (Table 6). Restricted to a constant and reflex impairment, the model predicted 79.4% of the results correctly. Cross-validation for *C. opilio* with a random learning set resulting in 80.4% correct predictions for the test set. Consequently, models for both species are robust for the experiments. Curves of the calculated probabilities of mortality based upon the logistic models with specific reflex impairments revealed very similar patterns for the two crab species (Fig. 6). Both plots rise in smooth sigmoid form from 13% to 17% mortality in crabs with impairments equal to zero to near total mortality with impairments equal to six.

Table 6

Results of logistic modeling for mortality in *Chionoecetes opilio* (snow crab). A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model.

Parameter	Estimate	*Z*	*P* value
Full model			
constant	–4.135	–1.323	0.186
temperature	0.644	1.088	0.277
crab size	0.015	0.426	0.670
reflex impairment	0.926	5.298	<0.001
Most parsimonious model			
constant	–1.868	–3.972	<0.001
reflex impairment	0.864	5.288	<0.001

Prediction matrix for the most parsimonious model

	Dead predicted	Live predicted	Actual total
Die	50	12	62
Live	9	31	40
Total no. predicted	59	43	102
Correct (%)	84.7	72.1	
False (%)	15.3	27.9	
Total correct (%)			79.4

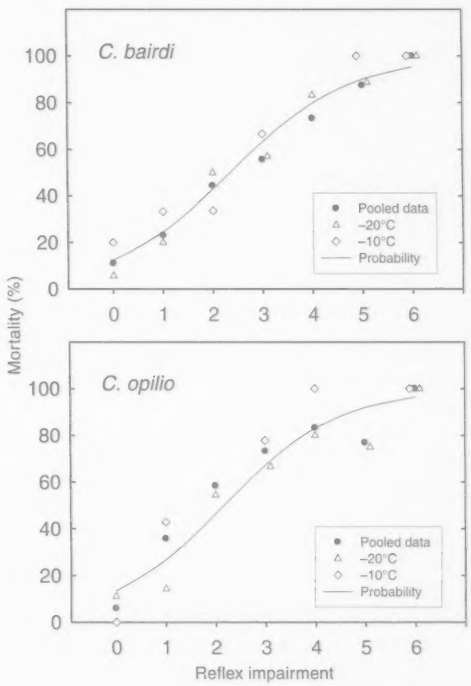

Figure 6

Relationships between percent mortality of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) and reflex impairment. Results are shown for exposure treatments made at two different temperatures where at least four crabs represent a point (open symbols) (see Fig. 5) and for pooled data (closed circles). The fitted curves represent results of logistic regression, and show the probabilities of mortality for the two crab species with varied levels of reflex impairment.

When reflexes were reassessed at the end of the 9-day holding period average impairments had decreased (i.e., condition improved) since the first assessment. This was true for both crab species and for both –20° and –10°C temperature treatments. However, most of the crabs with high initial reflex impairments died. More importantly, most individuals demonstrated either no change in reflex actions (85.1% of *C. bairdi*; 94.9% of *C. opilio*) or improvement during the holding period, and only a few individuals showed a one point increase in impairment. None of the control crabs died in holding and all had perfect reflex scores at the end of the experiment.

Discussion

Cold exposure and mortality

Although numerous studies have explored the effects of air exposure on economically significant crustaceans (e.g., Brown and Caputi, 1983; Vermeer, 1987; DiNardo et al., 2002; Lorenzon et al., 2007), and fishes (Olla et al., 1998; Davis et al., 2001; Gingerich et al., 2007) few experiments have been conducted to evaluate the effects of freezing temperatures relevant to high-latitude fisheries. In the present study, exposure to cold air caused high levels of mortality in *C. bairdi* and *C. opilio*, and the relationships to degree-hours of exposure were similar to those observed by Carls and O'Clair (1995) for *C. bairdi*. They reported a median lethal exposure for *C. bairdi* equal to –3.3°h for juveniles (46–74 mm CW) and –4.3°h for adult females (85–124 mm CW). The latter value corresponds closely with a median of approximately –4.8°h for male *C. bairdi* and –4.3°h for male *C. opilio*. Although the degree-hour exposure metric used in this and earlier studies provides a good integration of temperature and time, it is also clear that variation in mortality occurs with animal size (Carls and O'Clair, 1995), wind chill effect (Warrenchuk and Shirley, 2002), test temperature (this study), and other environmental variables (van Tamelen, 2005). Furthermore, large differences in mortality rates for *C. opilio* between this study and that conducted by Warrenchuk and Shirley (2002) illustrate the importance of specific environmental conditions. For example, in their study only five minutes exposure to windchill of –16°C (–1.33°h) resulted in 100% mortality. In comparison, the closest

test equivalent in this new study (–2°h) resulted in no mortality. Van Tamelen (2005) pointed out that simple wind chill estimates are not particularly useful in the context of heat exchange for crabs and he developed a thermal model for *C. opilio* that accommodates crab size and incorporates the numerous elements of chilling, such as convection, radiation, evaporation, and conduction.

Air exposure itself probably did not cause death. Earlier observations indicate that both *C. bairdi* and *C. opilio* are highly tolerant of handling and air exposures above freezing (MacIntosh et al., 1996; Grant, 2003). For example, McLeese (1968) observed no mortality in *C. opilio* after 4-day exposures to air in two above-freezing temperatures (3° and 8°C). Stevens (1990) reported a median lethal holding time of 8.3 hours in air for *C. bairdi*, and preliminary experiments at the Kodiak Laboratory (Stoner and Munk, unpubl. data) showed that *C. bairdi* could recover and survive for months after air exposures (15–18°C) up to seven hours. Although it is possible that mortality was caused by impaired oxygen delivery because of freeze-damaged gills, Carls and O'Clair (1995) argued convincingly against that mechanism, and Warrenchuk and Shirley (2002) proposed that mortality associated with freezing temperatures was caused by neurological damage. Their conclusion arose from observations of jerky, uncoordinated, and uncontrolled motions that would indicate nerve damage. Crabs in this study also commonly presented the same symptoms.

Autotomy

Shedding damaged limbs (autotomy) is an adaptation triggered by stress detectors in the limbs of crustaceans to prevent blood loss (Wales et al., 1971). The long, narrow form of the walking legs results in relatively rapid cooling (van Tamelen, 2005), and autotomy is commonly observed in *Chionoecetes* spp. exposed to freezing temperatures. In the present study, autotomy increased with increasing cold exposure, especially in *C. opilio*—a reaction analogous to that of earlier experiments with Alaskan crabs (Carls and O'Clair, 1995; Warrenchuk and Shirley, 2002). The proportions of types of limbs lost varied substantially between the two subject species for unknown reasons; however, the distribution of losses by *C. opilio* was similar to observations reported by Warrenchuk and Shirley (2002) who noted the relatively rare loss of chelipeds.

Delays in limb autotomy appear to be common among crabs and may have important consequences. As with the present study, others have observed that limb loss continues in holding tanks after exposure to cold temperatures (Carls and O'Clair, 1995; Warrenchuk and Shirley, 2002). The problem is exacerbated when limb losses increase during subsequent molting cycles (Carls and O'Clair, 1995). Given the apparent delays in autotomy, it is likely that limb losses observed before discard in typical fishing operations are substantially lower than the actual number. Autotomy may be especially critical for crabs that have reached a terminal molt or that molt infrequently. For example, *C. opilio* >90 mm CW appear not to regenerate limbs, and smaller crabs do so slowly, over at least two molt cycles (Miller and Watson, 1976). Therefore, Warrenchuk and Shirley (2002) considered autotomy to be a permanent injury for large *C. opilio*, and multiple limb losses create an obvious impediment for crabs returning to the bottom with regard to feeding, predator avoidance, reproductive behavior, and other ecological functions (see below).

Behavioral and reflex predictors of mortality

Righting ability depends upon a complex integration of neurological and mechanical systems, and several biologists have suggested that this may be a good condition index for Alaskan crabs (Stevens, 1990; Carls and O'Clair, 1995; Zhou and Shirley, 1995). As earlier, crabs demonstrating positive righting results in this study survived in high proportion. However, righting was a poor predictor of mortality because more than half of surviving crabs (both species) made no attempt to right. Also, Warrenchuk and Shirley (2002) found that the ability of crabs to right often recovered after several days. Although Stevens (1990) suggested that the time required to right might provide a useful predictor of survival, no such association was found in this study. Observation of righting behavior has two further limitations for assessing crab condition. First, because exposure to freezing temperatures and other forms of injury frequently result in autotomy, righting ability can be impaired by the lack of certain limbs. Second, the use of tanks of water to observe righting behavior on a moving vessel can be difficult or impractical.

Various other indicators have been used to assess the condition of crustaceans toward the goal of predicting survival or mortality. Stevens (1990) evaluated the vitality of Alaskan crabs, scoring them as *alive and active*, *moribund*, or *dead*. Purves et al. (2003) used a similar three-tier index of vitality (i.e., *lively*, *limp*, or *dead*) in three lithodid crabs (*Paralomis* spp.) to evaluate how different fishing modes used in the South Atlantic toothfish (*Dissostichus eleginoides*) fishery affect the bycatch mortality of crabs. Criteria for the index incorporated four reflexes, namely actions by the mouth parts, chelae, and legs, and leg flare. Therefore, the vitality index used by Purves et al. (2003) could have been expanded to a reflex impairment score with five increments (0–4) instead of just two for live crabs. The reflex impairment score reported in this study was based upon the same principle of considering each reflex independently and providing a seven-increment resolution (0–6).

Davis (2007) and Stoner et al. (2008) discussed the merits of using multiple reflexes and a higher resolution for a reflex impairment index to predict discard mortality. One advantage is that the composite score reflects animal condition and the likelihood of survival over a wide range of stressor types and environmental exposures (i.e., physiological stress as well as physical injuries). This result is true because different forms of stress can have different impacts on the reflexes tested,

but considered together, they reflect overall condition. For example, chela closure was the most sensitive indicator of freezing stress observed in this study, but chela function was affected in only 14% of crabs (*C. bairdi* and *C. opilio*) injured in trawl capture (Stoner et al., 2008). Impairment of reflex action in eye stalks was also elevated in crabs exposed to freezing temperatures but was rare in trawl-captured crabs. Conversely, leg retraction and leg flaring actions were impaired infrequently under freezing temperatures but were commonly impaired in trawl-captured crabs. Despite these differences, mortality was closely associated with reflex impairment in both the freezing-stress and trawl-stress experiments, and the logistic regressions for *C. bairdi* and *C. opilio* in this study were nearly identical. This result indicates that the reflex impairment score is a robust tool for predicting mortality in *Chionoecetes* species.

Limitations of reflex action mortality predictors

The RAMP approach does have certain limitations because, as with other measures of animal condition, some of the indirect and delayed effects of the discarding process and the behavioral impairments were not considered. 1) Predation on discarded catch can occur in the water column. Marine mammals, large fish, and birds often follow fishing vessels, scavenging injured and uninjured discards. 2) Impairments in defensive behaviors can result in predation once a crab has reached its benthic habitat. Some of these discards would otherwise survive their injuries. 3) Impairments in sensory apparatus, feeding appendages, or locomotory functions (e.g., because of limb damage or autotomy), can result in starvation. Carls and O'Clair (1995) showed that sublethal effects of freezing on *C. bairdi* can include reduced feeding rate and growth. In addition, mortality observed in laboratory experiments may not reflect the lost function of sensory appendages that aid in locating food or avoiding predators. Eye stalks are particularly susceptible to freezing (van Tamelen, 2005), but it is unknown how vision may be impaired by exposure of crabs to freezing temperatures. The eyes of deep-dwelling decapods can also be damaged by exposure to sunlight (Gaten 1988). Future experiments should be designed to test for losses in vision, chemoreception, and other sensory functions after exposures of crabs to air and freezing temperature. 4) Freezing temperatures can also cause long-term injury resulting in unsuccessful molting (O'Brien et al., 1986). Carls and O'Clair (1995) reported increased limb loss at molting after exposure to freezing, and deaths during molting are a common occurrence in Alaskan crabs (Stoner, pers. observ.). 5) Freezing and other forms of injury may also raise the animal's susceptibility to disease.

RAMP curves as currently developed do not account for these forms of discard mortality, and do not provide an absolute value for discard mortality. However, it is likely that the last four sources of mortality discussed above will be directly proportional to the reflex impairments observed. Longer term holding or tagging experiments could provide greater insight into the relationships between reflex impairment and long-term survival of discarded animals.

Conclusions

Fisheries for *Chionoecetes* spp. in Alaska are centered on the winter season, and the threat of cold-related mortality in the face of wind chill conditions is real. One could employ van Tamelen's (2005) thermal model to obtain crab mortality estimates in the field; however, this would require continuous monitoring of a wide array of environmental conditions as well as crab measurements. In addition, mortality of crabs in the field results from various combinations of physiological stressors and physical injuries (not only thermal stress) for which appropriate mortality rates are not known. A simpler and more direct measure of crab condition and viability is provided by the RAMP approach. Impaired reflexes reveal stress and the composite reflex impairment index allows a calculated probability of mortality that is independent of crab size, physical injuries, and exposure conditions (Stoner et al., 2008; this study). Representative crabs are observed in hand, the reflex actions are summed, and probability of mortality for an individual or a treatment population can be extracted quickly from the RAMP curve with reasonable precision. Once a robust RAMP curve is established, presence or absence of reflex actions are the only observations required to calculate probability of mortality. The approach should be equally applicable to the wide variety of crabs, lobsters, and shrimps that are routinely discarded as unwanted or illegal bycatch, or in instances where an immediate assessment of animal condition or discard mortality is required. Greatest use for the RAMP approach will occur in experiments with fishing gear or handling methods aimed at reducing bycatch or discard mortality.

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Diet composition and prey selection of the introduced grouper species peacock hind (*Cephalopholis argus*) in Hawaii

Jan Dierking (contact author)[1,2]
Ivor D. Williams[3,4]
William J. Walsh[3]

E-mail address for contact author: jan.dierking@univmed.fr

[1] Department of Zoology
University of Hawaii
2538 The Mall
Honolulu, Hawaii 96822

[2] Present address: Centre d'Océanologie de Marseille, UMR CNRS 6540
Université de la Méditerranée, Station Marine d'Endoume
Rue de la Batterie des Lions
13007 Marseille, France

[3] Hawaii Division of Aquatic Resources, Honokohau Marina
74-380B Kealakehe Pkwy
Kailua-Kona, Hawaii 96740

[4] Hawaii Cooperative Fishery Research Unit
Department of Zoology
University of Hawaii at Manoa
Honolulu, Hawaii 96822

Abstract—The introduced grouper species peacock hind (*Cephalopholis argus*), was the dominant large-body piscivore on the Main Hawaiian Island (MHI) reefs assessed by underwater visual surveys in this study. However, published data on *C. argus* feeding ecology are scarce, and the role of this species in Hawaiian reef ecosystems is presently not well understood. Here we provide the first comprehensive assessment of the diet composition, prey electivity (dietary importance of prey taxa compared to their availability on reefs), and size selectivity (prey sizes in the diet compared to sizes on reefs) of this important predator in the MHI. Diet consisted 97.7% of fishes and was characterized by a wide taxonomic breadth. Surprisingly, feeding was not opportunistic, as indicated by a strongly divergent electivity for different prey fishes. In addition, whereas some families of large-body species were represented in the diet exclusively by recruit-size individuals (e.g., Aulostomidae), several families of smaller-body species were also represented by juveniles or adults (e.g., Chaetodontidae). Both the strength and mechanisms of the effects of *C. argus* predation are therefore likely to differ among prey families. This study provides the basis for a quantitative estimate of prey consumption by *C. argus*, which would further increase understanding of impacts of this species on native fishes in Hawaii.

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The grouper species peacock hind (*Cephalopholis argus*) (Serranidae: Epinephelinae), a reef fish predator that is native over most of the Indo-Pacific region, was introduced to the Main Hawaiian Islands (MHI) in 1956 as part of an introduction program of snapper and grouper species intended to enhance nearshore fisheries (Randall, 1987). Today, this non-native predator occupies a dominant position in the guild of large piscivores on many reefs in the MHI (see *Results* section). As impacts of piscivores on prey fishes have been demonstrated in a number of studies (e.g., Webster, 2002; Hixon and Jones, 2005), the abundance of *C. argus* raises the question of how, if at all, it affects native reef fishes in Hawaii. However, the only published study of *C. argus* feeding in the MHI to date was based on a sample of 10 specimens (Hobson, 1974), which is insufficient to elucidate feeding patterns.

Groupers are among the most common predatory reef fishes worldwide (Parrish, 1987). They play an important role in shaping reef communities (Goeden, 1982; Parrish, 1987) and are of large commercial importance in coral reef fisheries (Heemstra and Randall, 1993). However, data on the general feeding ecology of this family remain surprisingly scarce (Beukers-Stewart and Jones, 2004). In addition, although concern about declines of many grouper species worldwide due to overfishing has led to a renewed research focus on this family (Morris et al., 2000), much of the work to date has concentrated on a limited number of species, e.g., the coral trout (*Plectropomus leopardus*) in Australia (e.g., Kingsford, 1992; St. John, 1999), the Nassau grouper (*Epinephelus striatus*) in the Caribbean Sea (e.g., Eggleston et al., 1998), or the dusky grouper (*Epinephelus marginatus*) in the Mediterranean Sea (e.g., Renones et al., 2002).

Most studies of grouper diet have been based on analysis of stomach contents. However, because of the difficulty of obtaining large grouper samples (Beukers-Stewart and Jones, 2004), as well as the high prevalence of empty stomachs due to prey regurgitation during capture and because of the characteristics of grouper feed-

ing ecology (Dierking and Meyer, in press), detailed descriptions of grouper diet remain rare. Notably, only one study of *C. argus* diet (Randall and Brock, 1960), and a few studies of other grouper species (but see Kingsford, 1992; St. John, 1999; Beukers-Stewart and Jones, 2004), have been based on more than 50 full stomachs. Interpretation of feeding patterns is further complicated by the lack of studies comparing dietary composition with prey availability in the wild (but see Beukers-Stewart and Jones, 2004).

We examined the feeding patterns of *C. argus* based on stomach content analysis of the largest sample of this species (n=285) available to date. In addition, we assessed the patterns in the context of the composition of the reef fish assemblage in Hawaii, which was determined by underwater visual surveys. The main goal was to describe the diet composition, prey electivity (dietary importance of a taxon compared to its availability on reefs), and size selectivity (prey sizes in the diet compared to sizes on reefs) of *C. argus* in Hawaii. Secondary goals were to assess the mechanisms by which this non-native predator may affect prey fishes and to provide data required for the quantitative estimation of prey consumption by this species.

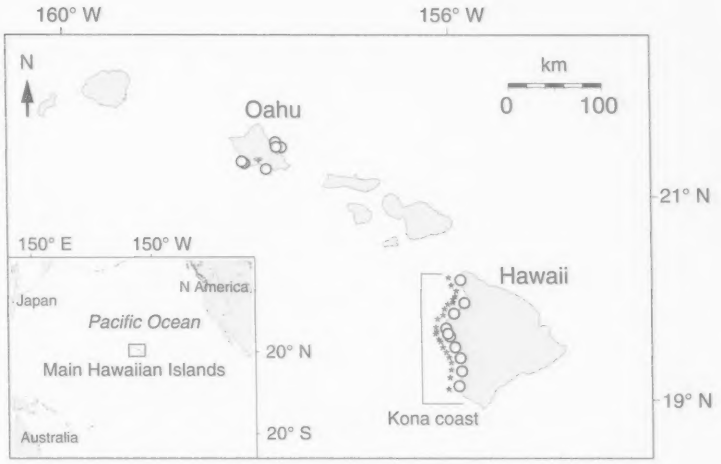

Figure 1

Map of the Main Hawaiian Islands, with peacock hind (*Cephalopholis argus*) collection sites marked by open circles, and underwater visual survey sites along the Kona coast marked by asterisks (Note: asterisks were moved offshore from actual survey locations to avoid overlap with sample site symbols).

Material and methods

Study organism and sampling sites

To our knowledge, the establishment of *C. argus* in Hawaii represents the first documented case of the successful invasion of a non-native habitat by a grouper. Today this generally piscivorous species (Parrish, 1987) is found around all of the MHI. It is particularly abundant along the western coastline of the island of Hawaii (Kona coast hereafter, following common terminology in Hawaii), which harbors some of the least disturbed reefs in the MHI and is the source of important economic revenues from diving tourism and the aquarium fish industry (Tissot et al., 2004). Despite its abundance, a fishery for *C. argus* never developed because it turned out to be a carrier of ciguatoxin, the agent of ciguatera fish poisoning (Dierking and Campora, 2009).

Cephalopholis argus specimens from the Kona coast (n=179, 11 sites) and from the island of Oahu (n=106, 6 sites) (Fig. 1) were obtained by spearfishing with scuba in July 2003. Divers attempted to spear all sighted individuals regardless of size or behavior pattern (e.g., active swimming, resting). Collections took place between 0924 and 1522 hours at a mean depth of 11.6 m. Speared specimens were immediately (i.e., underwater) sealed in plastic bags to avoid stomach content loss from regurgitation commonly observed in groupers (Dierking and Meyer, in press). In the laboratory, standard length (SL) and total length (TL) (equal to fork length in *C. argus* due to their rounded caudal fin shape) were recorded to the nearest mm. Wet mass (M) of *C. argus* from the Oahu sites was measured to the nearest 5 g. Based on these measures, morphometric relationships (SL-M, TL-M, SL-TL) (Table 1) were calculated. The SL-M equation was then used to estimate the wet mass of Kona specimens, which could not be measured in the field owing to scale malfunctioning.

Table 1

Morphometic relationships between total length (TL), standard length (SL, in cm), and wet mass (M, in g) for peacock hind (*Cephalopholis argus*) in Hawaii. r^2 = regression fit.

Relationship	a	b	n	r^2
$M = a\ TL^b$	0.0125	3.122	110	0.98
$M = a\ SL^b$	0.0309	3.013	110	0.97
$SL = a + b\ TL$	−0.244	0.8494	304	0.99

Diet composition

To determine the diet composition of *C. argus*, stomachs of all specimens were opened and any prey items were removed. The analysis of contents then followed the procedures described by Hyslop (1980). Specifically, for

the two island samples and the combined overall sample, the vacuity rate (i.e., the proportion of empty stomachs) was determined. Differences in vacuity between islands were assessed with a chi-square test. For full stomachs, the number of prey items was counted, and the SL and TL of fish prey and the carapace length of crustacean prey were determined to the nearest mm, where digestion state allowed reliable measurements. For fishes, SL could be determined more often than TL because skeletons are slow to disintegrate during digestion. In these cases, TL was calculated from SL according to published SL-TL equations for the respective taxon (Froese and Pauly, 2009). The M of all prey items was recorded to the nearest mg. Mean relative stomach content M was then calculated as total M of prey items divided by the total M of *C. argus* specimens.

We identified each prey item to the lowest possible taxonomic level, using Randall's (1996; 2007) key for fish prey, and Hoover's (1998) key for crustacean prey. Cumulative prey curves (Ferry and Cailliet, 1996) derived from plotting the cumulative number of unique prey taxa against the cumulative number of analyzed stomachs allowed us to assess whether sample sizes were large enough to accurately characterize dietary breadth. These curves reach an asymptote if sample size is sufficient. To determine dietary importance of prey, for all identified prey types and families, the numerical importance (*%N*), frequency of occurrence (*%O*) (calculated from full stomachs), and gravimetric importance (*%M*), as well as the index of relative importance (*IRI*) and the *%IRI* (proportion of the *IRI* of a taxon to the sum of *IRI*s of all taxa) were calculated. The *IRI* incorporates the individual indices in the formula

$$IRI = (\%N + \%M) \times \%O \quad (1)$$

and may provide a more accurate description of dietary importance than its components by canceling out their individuals biases, such as the overestimation of the importance of an abundant but small prey item by the *%N* (Cortes, 1996). Because *%N*, *%M*, *%O*, and *%IRI* indicated that fishes dominated the diet of *C. argus*, for the comparison of diets between islands and the calculation of electivity, the indices were recalculated for the fish component of the diet alone.

Composition of the reef fish assemblage

Underwater visual surveys with scuba were used to determine reef fish abundances and sizes (in 5-cm bins, i.e., 0–5 cm, 5–10 cm, etc.) at 23 sites (depth range 8.2 m–18.2 m, mean depth 11.9 m). All sites were located in the dominant reef habitat of the Kona coast, reef shelves with moderate to high finger coral (*Porites compressa*) cover. Each survey of a site involved four divers (two pairs), who between them surveyed four 25×4 m (100 m^2) belt transects that were permanently installed at each site. Each transect count consisted of one rapid swim to count mobile and midwater species, and a slow return swim closer to the bottom to record fishes in and around the benthos. Sites were surveyed 4 to 6 times during the year 2003, generally between 0840 and 1600 hours. The detailed sampling regime was described by Tissot et al. (2004). Surveys were conducted under the direction of the West Hawaii Aquarium Project (WHAP, a collaboration of the Hawaii Division of Aquatic Resources (HDAR), the University of Hawaii at Hilo, and Washington State University), and are therefore referred to as "WHAP surveys" here.

WHAP survey counts were used to calculate mean fish densities (individuals/100 m^2) and relative numerical importance (*%N*) of reef fishes in Kona in 2003 (i.e., grand mean of densities for the year 2003 at the 23 sites). In addition, relative importance in terms of biomass (*%M*) was determined for large (mean body M >50 g) piscivores. For this purpose, the M of individuals was estimated from their TL by using conversion equations for the respective taxa (Froese and Pauly, 2009). Finally, size-frequency distributions of reef fishes in Kona were calculated from the combined WHAP survey counts for 2003.

Abundances of nocturnally active taxa tend to be underestimated by data collected during daytime surveys (Ackerman and Belwood, 2000). For the calculation of electivity (see next section), abundances of the nocturnal apogonids, holocentrids, and priacanthids were therefore estimated by nighttime surveys ("Night WHAP") that took place in 2003 at the same sites surveyed during daytime. The ratios of nighttime to daytime abundances for these families were 90.6, 2.6, and 1.5, respectively.

Prey selection

To determine the taxonomic focus of predation, we used Ivlev's electivity index (Ivlev, 1961):

$$E_i = (r_i - p_i) / (r_i + p_i), \quad (2)$$

where r_i = numeric importance (*%N*) of fish family *i* in the diet of *C. argus*; and
p_i = *%N* of the same family in the reef environment.

E_i can take values between –1 and 1. Positive values indicate "preference" (a taxon overrepresented in the diet in relation to its availability in the environment), and negative values "avoidance" (a taxon underrepresented in the diet in relation to its availability) (Lechowicz, 1982). Because of the scarcity of reef fish abundance data for Oahu for the year 2003, when stomach contents for this study were obtained, we based electivity calculations on diet composition data obtained from the Kona sample, and on reef fish abundance data for the Kona coast from WHAP surveys in 2003.

Prey-size selection

To assess the size focus of *C. argus* predation, length-frequency distributions of important fish families in the diet of *C. argus* were compared with length-frequency

Table 2

Diet composition of peacock hind (*Cephalopholis argus*) in Hawaii, based on Kona and Oahu samples combined (n_{total}=285, $n_{full\ stomachs}$=159), by number (N), occurrence (i.e., number of stomachs in which a taxon occurred) (O), and mass (M, in g). Dietary importance is indicated by percent by number (*%N*), percent by occurrence (*%O*) (calculated on the basis of full stomachs), percent by mass (*%M*), and percent index of relative importance (*%IRI*, based on the index of relative importance *IRI* = (*%N* + *%M*) ×*%O*). *%O* and *%IRI* for fishes and crustaceans are nonadded values (i.e., they correspond to these two food types, not the sum of their components). Unidentified fish and crustacean prey were excluded from family-level calculations of % indices.

Prey taxon	N	O	M	*%N*	*%O*	*%M*	*%IRI*
Fish	185	144	1346.0	84.5	90.6	95.5	97.7
Acanthuridae	14	13	137.8	12.3	8.2	11.9	17.3
Acanthurus nigrofuscus	3	3	11.2				
Acanthurus nigroris	2	2	89.8				
Zebrasoma flavescens	2	2	18.7				
unidentified Acanthuridae	7	6	18.2				
Apogonidae	3	3	15.3	2.6	1.9	1.3	0.7
unidentified Apogonidae	3	3	15.3				
Aulostomidae	6	6	29.4	5.3	3.8	2.5	4.3
Aulostomus chinensis	6	6	29.4				
Balistidae	3	3	63.5	2.6	1.9	5.5	1.3
Xanthichthys auromarginatus	1	1	53.5				
unidentified Balistidae	2	2	10.0				
Chaetodontidae	6	6	78.6	5.3	3.8	6.8	4.0
Chaetodon multicinctus	1	1	16.9				
Forcipiger flavissimus	3	3	21.8				
Hemitaurichthys polylepis	1	1	24.8				
unidentified Chaetodontidae	1	1	15.1				
Cirrhitidae	4	4	37.7	3.5	2.5	3.3	1.5
Amblycirrhitus bimacula	1	1	4.6				
unidentified Cirrhitidae	3	3	33.1				
Holocentridae	16	16	52.6	14.0	10.1	4.6	16.3
Sargocentron punctatissimum	1	1	4.0				
unidentified Holocentrinae	15	15	48.6				
Kuhliidae	1	1	17.5	0.9	0.6	1.5	0.1
Kuhlia spp.	1	1	17.5				

continued

distributions of the same taxa in the reef environment in Kona, by using 2-sample Kolmogorov-Smirnov tests. The family Priacanthidae was excluded from the analysis despite its dietary importance because low counts in underwater surveys did not allow for meaningful comparison. Minitab 14 software (Minitab Inc., State College, PA) was used for all statistical analyses, and results were considered significant at $P<0.05$.

Results

Morphometrics of *C. argus*

Cephalopholis argus SL ranged from 13.2 to 44.0 cm (mean: 26.9 cm), and M from 69 g to 2847 g (mean: 721 g). Morphometric relationships (M-TL, M-SL, SL-TL), which have not been reported for this species from large sample sizes, are summarized in Table 1.

Diet composition

The stomach vacuity rate for the overall sample of 285 analyzed stomachs was 44.2%, and the mean relative stomach content M was 0.74% of *C. argus* body M (empty stomachs included in the calculation). Overall, 219 prey items were recovered from 159 full stomachs. Reef fishes were the principal diet component (97.7% by *%IRI*). Crustaceans were the only other higher taxonomic group in the diet, but were of minor importance (2.3% by *%IRI*) (Table 2). Dietary breadth was wide; a total of 24 prey species (20 fish species) in 20 different prey families (16 fish families) were found in the diet. At the same time, the three most important families in the diet made up almost 60%, and the eight most important fish families close to 90% of the total diet (by *%IRI*). In declining order of importance, these families were the Scaridae, Acanthuridae, Holocentridae (exclusively of the subfamily Holocentrinae, the squirrelfishes), Monacanthidae,

Table 2 (continued)

Prey taxon	N	O	M	%N	%O	%M	%IRI
Labridae	2	2	29.5	1.8	1.3	2.6	0.5
Pseudocheilinus tetrataenia	1	1	1.7				
Stethojulis balteata	1	1	27.8				
Monacanthidae	15	13	52.5	13.2	8.2	4.5	12.6
Pervagor aspricaudus	2	2	19.5				
unidentified Monacanthidae	13	11	33.0				
Mullidae	3	3	40.3	2.6	1.9	3.5	1.0
Unidentified Mullidae	3	3	40.3				
Pomacanthidae	1	1	19.8	0.9	0.6	1.7	0.1
Centropyge potteri	1	1	19.8				
Pomacentridae	3	3	35.4	2.6	1.9	3.1	0.9
Stegastes marginatus	2	2	29.1				
unidentified Pomacentridae	1	1	6.3				
Priacanthidae	10	8	125.1	8.8	5.0	10.8	8.6
Heteropriacanthus cruentatus	9	7	122.2				
unidentified Priacanthidae	1	1	2.9				
Scaridae	12	12	319.0	10.5	7.5	27.6	25.1
Calotomus carolinus	1	1	72.0				
Scarus psittacus	4	4	107.1				
unidentified Scaridae	7	7	139.9				
Synodontidae	1	1	41.6	0.9	0.6	3.6	0.2
Saurida gracilis	1	1	41.6				
Unidentified fishes	85	70	250.4				
Crustaceans	34	29	78.6	15.5	18.2	5.5	2.3
Grapsidae	1	1	9.0	0.9	0.6	0.8	0.1
Plagusia depressa	1	1	9.0				
Hippolytidae	10	10	38.1	8.8	6.3	3.3	6.6
Saron marmoratus	10	10	38.1				
Portunidae	2	2	12.0	1.8	1.3	1.0	0.3
Charybdis hawaiensis	1	1	10.6				
Charybdis paucidentata	1	1	1.4				
Rhynchocinetidae	1	1	0.3	0.9	0.6	0.0	0.0
unidentified Rhynchocinetidae	1	1	0.3				
Unidentified crustaceans	20	18	19.2				
Subtotal (unidentified fishes and crustaceans excluded)	114	109	1154.9	100.0	108.8	100.0	100.0
Total (all prey items)	219	197	1424.5				

Priacanthidae, Chaetodontidae, Aulostomidae, and Cirrhitidae (Table 2).

Oahu and Kona *C. argus* populations did not differ significantly in either stomach vacuity rate (50.0% vs. 40.7%; chi-square test, n=285, P=0.13) or relative stomach fullness (0.73% vs. 0.76% of own body M; t-test, n=285: P=0.84). Both island populations also revealed the importance of fish prey in their diet (96.7% vs. 98.1% by *%IRI*) and showed a similar overall dietary breadth (14 versus 18 families). In addition, the Scaridae, Acanthuridae, and Priacanthidae were concurrently among the five most important prey families by *%IRI* for both islands (Table 3). Still, diet composition differed in several respects. Most importantly, the dominant prey family by *%IRI* in the diet of *C. argus* from Kona (Holocentridae) was not found in the diet of *C. argus* from Oahu, and vice versa, the dominant family in the diet of Oahu *C. argus* (Monacanthidae) was rare in the diet of *C. argus* from Kona. The only other family for which *%IRI* values differed by >5% was the Chaetodontidae, which was more important in the Kona than the Oahu diet. Balistidae and Mullidae were slightly more important in the Kona diet, and Pomacentridae, Cirrhitidae, and Synodontidae in the Oahu diet. For all remaining families, absolute *%IRI* values differed by <1% (Table 3).

Cumulative prey curves for family-level analyses based on the overall (i.e., Oahu and Kona samples combined) and on the Kona sample showed strong asymp-

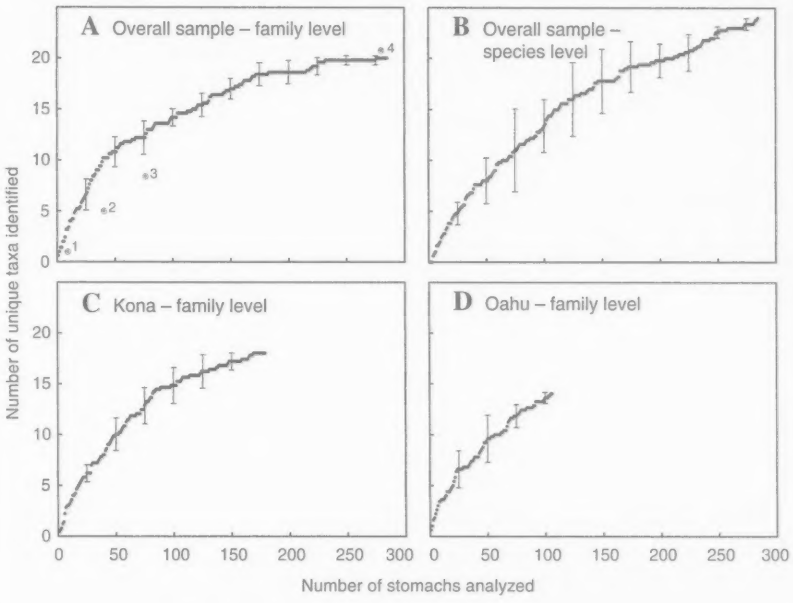

Figure 2

Cumulative prey curves for the assessment of sample-size sufficiency in characterizing dietary breadth. "Overall sample" refers to the combined Oahu and Kona *Cephalopholis argus* samples (n=285). In each panel, A–D, the number of distinct taxa identified in the diet is plotted against the number of stomachs analyzed. The approximation of curves to an asymptote indicates that sample size was sufficiently large. The order of samples was randomized five times, and the mean number of unique prey items and the standard deviation for every 25th sample were calculated from the five orders of analysis. The four added data points in panel A (◉) show results of previous studies on *C. argus* (1=Hobson 1974; 2=Randall 1980; 3=Shpigel and Fishelson 1989a; 4=Randall and Brock 1960). Sample size and number of identified taxa for these studies are strongly and significantly correlated (Pearson's r=0.97, P=0.03).

totic behavior (Fig. 2, left panels), which indicates that sample size was sufficiently large to describe dietary breadth. In contrast, curves for the species-level analysis based on the overall sample and for the family-level analysis based on the Oahu sample (Fig. 2, right panels) showed only a slight approximation to an asymptote, and dietary breadth may therefore be incompletely described at these levels.

Composition of the reef fish assemblage

Figure 3 summarizes the mean densities of the 32 different families of reef fishes observed on Kona reefs during the 2003 WHAP surveys. The families Acanthuridae and Pomacentridae, with densities of 69.2 and 61.8 individuals/100 m^2, together accounted for almost 75% of the total fish assemblage in terms of %*N*. Seven other families contributed at more than 1% by %*N*, the most important ones being the Labridae (9.0%) and Chaetodontidae (4.6%). The importance of nocturnally active families may be higher than shown in Figure 3, which is based on daytime WHAP surveys alone. In particular, recalculated numerical importance for the nocturnal families Apogonidae, Holocentridae, and Priacanthidae (based on nighttime surveys showing a 90.6, 2.6, and 1.5 times higher abundance than during daytime; see *Material and methods* section) would be 7.5%, 6.8%, and <0.1%, respectively. The guild of large-body piscivores contributed 0.7% to total fish abundance. Within this guild, *C. argus* (density=0.70 individuals/100 m^2) was the dominant taxon, contributing 56% by %*N* (Fig. 3) and 84% by %*M*—the larger value for the latter being due to a higher mean body M for *C. argus* compared to the other predators in the analysis.

Prey selection

All of the 10 most abundant species on Kona reefs were found in the stomachs of *C. argus*. However, although some fishes that were rare in the reef environment

Table 3

Composition of the fish portion of the diet of peacock hind (*Cephalopholis argus*) from Hawaii Island (n=179) and Oahu (n=106), based on fish prey identified to at least the family level. Crustaceans were excluded from the analysis because of their relatively minor dietary importance for *C. argus* from both islands (*%IRI* =1.9% and 3.3%, respectively). Dietary importance is indicated by percent by number (*%N*), percent by occurrence (*%O*) (calculated on the basis of full stomachs), percent by mass (*%M*), and percent index of relative importance (*%IRI*).

	Island							
	Hawaii				Oahu			
Family	*%N*	*%O*	*%M*	*%IRI*	*%N*	*%O*	*%M*	*%IRI*
Acanthuridae	16.9	9.4	13.0	20.9	8.6	5.7	11.9	7.1
Apogonidae	3.1	1.9	1.2	0.6	2.9	1.9	1.7	0.5
Aulostomidae	6.2	3.8	3.1	2.6	5.7	3.8	2.0	1.8
Balistidae	4.6	2.8	9.1	2.9	—	—	—	—
Chaetodontidae	7.7	4.7	10.1	6.2	2.9	1.9	2.1	0.6
Cirrhitidae	3.1	1.9	0.9	0.6	5.7	3.8	7.9	3.2
Holocentridae	24.6	15.1	7.5	35.8	—	—	—	—
Kuhliidae	1.5	0.9	2.5	0.3	—	—	—	—
Labridae	1.5	0.9	0.2	0.1	2.9	1.9	7.0	1.1
Monacanthidae	6.2	2.8	1.6	1.6	31.4	18.9	10.3	48.6
Mullidae	4.6	2.8	5.8	2.2	—	—	—	—
Pomacanthidae	—	—	—	—	2.9	1.9	5.0	0.9
Pomacentridae	1.5	0.9	0.9	0.2	5.7	3.8	7.3	3.0
Priacanthidae	9.2	5.7	9.2	7.7	11.4	3.8	15.2	6.2
Scaridae	9.2	5.7	34.8	18.4	17.1	11.3	19.1	25.3
Synodontidae	—	—	—	—	2.9	1.9	10.5	1.6

("the wild") in Kona were important components of *C. argus* diet (e.g., Priacanthidae: $\%N_{reef\ environment}$ <0.1%, $\%N_{diet}$=9.2%), others such as the Pomacentridae, although highly abundant on Kona reefs (*%N*=31.5%), had low dietary importance (*%N*=1.5%) (Fig. 3, Table 2). Consequently, the electivity values of prey families ranged widely, from values of E_i near 1 (strong preference) to −0.91 (strong avoidance). This pattern was consistent both for diurnally and nocturnally active taxa (Fig. 4). Specifically, of the nocturnal taxa, priacanthids and holocentrids were highly preferred, whereas apogonids were avoided. Diurnally active families can be divided into three broad categories based on their electivity (Fig. 4) and their abundance on reefs (Fig. 3): 1) negative electivity, large wild abundance (Pomacentridae, Labridae, Acanthuridae); 2) moderate positive electivity, moderate to large wild abundance (e.g., Chaetodontidae, Mullidae, Scaridae); and 3) large positive electivity, low wild abundance (Aulostomidae, Monacanthidae).

Prey-size selection

The mean TL of reef fishes in the diet of *C. argus* was 7.2 cm, and was thus significantly smaller than the mean TL of reef fishes in the wild of 9.1 cm (Kolmogorov-Smirnov test, D=0.11, P=0.034) (Fig. 5). Mean size in the diet was consistently lower than mean size in the wild for all analyzed prey families. However, specific patterns differed strongly. On one end of the spectrum, for generally small-body families, such as the Acanthuridae and Chaetodontidae, differences between consumed and wild sizes were near significant ($D_{Acanthuridae}$=0.32, P=0.057) or significant ($D_{Chaetodontidae}$=0.55, P=0.026), but small in absolute terms, with size classes occurring in the diet overlapping strongly with those observed in the wild. The Scaridae (D=0.42, P=0.021) represented an intermediate case, with predation focused on the smaller size classes present in the wild, but clear overlap between size-frequency distributions in the diet and in the wild. Finally, for the generally large-body families Monacanthidae (D=0.47), Aulostomidae (D=0.82), and Holocentridae (D=0.99), size differences between the diet and the wild were large and highly significant (P<0.01), and sizes as small as in the diet were rarely observed in the wild (Fig. 5).

Discussion

The comparison of reef fish densities in this study showed that *C. argus* constituted more than half of the guild of large piscivores in Kona by numerical abundance, and more than 80% of this guild by biomass. Because consumption partly depends on the biomass of a predator (Cortes, 1996), it therefore appears safe to say that *C. argus* has become the dominant large-body predatory

reef fish in this area. Densities of *Cephalopholis argus* observed in Kona (0.70 ind./100 m^2) were lower than those in the Red Sea at shallow depths (1.32 ind./100 m^2), but much higher than in the Red Sea at depths >10 m, where other species of the genus *Cephalopholis* outcompete *C. argus* (Shpigel and Fishelson, 1989b). In addition, the density of *C. argus* was higher than that of the ecologically important grouper *Plectropomus leopardus* in Australia (0.53 ind/100 m^2) (St. John, 1999). In this context, knowledge of the feeding patterns of *C. argus* in Hawaii is particularly relevant because it provides the basis for the assessment of effects of *C. argus* on native species.

Diet composition was characterized by the large dietary importance of fishes (97.7% by *%IRI*), wide dietary breadth (4 crustacean and 16 fish families present in the diet), and a nearly exclusive focus on benthic reef-associated fishes. These patterns are typical for groupers, which are usually ambush predators that hunt close to the reef and prey on a wide range of different fishes, as well as crustaceans and, in some cases, cephalopods (Parrish, 1987). In *C. argus*, a strong focus on fish prey was also previously observed in locations outside the MHI (e.g., 77.5% by *%N* in the Society Islands [Randall and Brock, 1960]; 95.7% by *%M* in Madagascar [Harmelin-Vivien and Bouchon, 1976]; 92% by *%N* in the South Pacific [Randall, 1980]; 95% by *%N* in the Red Sea [Shpigel and Fishelson, 1989a]). Although *C. argus* is a non-native species in Hawaii, its feeding ecology thus appears to reflect the feeding ecology of the species in native habitats.

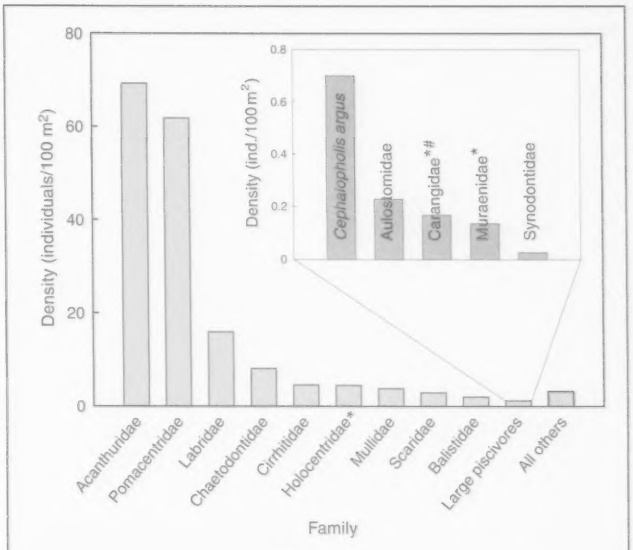

Figure 3

Densities of fish families in typical *Cephalopholis argus* habitat along the Kona coast (families contributing <1% to total fish abundance are summarized under "All others"), based on replicated daytime underwater visual surveys at 23 sites along this coast in 2003 (see Fig. 1). The inlay shows densities of families in the guild of large piscivores (mean mass >50 g) along the Kona coast. Notes: *=at least in part nocturnally active; importance therefore underestimated by daytime surveys. #=roving predator; abundance difficult to assess with the belt transects in this study.

Half of all fish families that inhabit reefs in Kona (32 families in 2003) were present in the diet of *C. argus*—a finding that demonstrates that groupers may prey on a large proportion of the fish taxa present in their habitat. The dietary breadth (fishes and crustaceans combined) observed in our study was much wider than those previously reported for *C. argus* in Hawaii (1 family observed in 10 samples [Hobson, 1974]), in the South Pacific (5 families in 39 samples [Randall, 1980], and in the Red Sea (8 families in 78 samples [Shpigel and Fishelson, 1989a]). In contrast, the dietary breadth equaled the breadth reported for the Society Islands (21 families in 280 samples [Randall and Brock, 1960]). Considering the small samples sizes of most previous studies, and the association of dietary breadth with sample size (see *Results* section on cumulative prey curves), differences were likely related to sample size and not to divergent feeding ecology. This interpretation is supported by the strong correlation of the number of identified taxa and sample sizes of previous studies (see added data points in Figure 2).

The similar vacuity rate, stomach fullness, importance of fish prey, and dietary breadth observed in the Kona and Oahu samples indicated that feeding ecology was consistent between those islands. The small differences in dietary importance observed for some families could be related to chance variation, in particular if occurrence of a family in the diet is infrequent. Although the lack of a reef fish survey program comparable to the WHAP program for Kona does not allow a direct comparison between Oahu and Kona reef fish assemblages, divergent dietary importance could also reflect variations in fish assemblages, for example, variations due to differences in reef habitat or fishing pressure. In particular, Williams et al. (2008) demonstrated that the abundance of fishery target species in the MHI is negatively correlated with local human population density, which is noteworthy in this context because population density on Oahu is more than 30-fold higher than on Hawaii Island. In contrast, the large differences observed for small individuals of the Holocentridae and Monacanthidae are probably related to local recruitment pulses, which is further discussed in the section on size selection below.

Prey selection

The wide range of electivity values for prey fishes in the diet of *C. argus* in this study indicates that feeding was

not opportunistic. This finding is surprising because groupers are often considered generalist, opportunistic predators on account of their wide dietary breadth (Parrish, 1987). However, the only other study in which the relative importance of prey fishes in the wild was compared with that in grouper diet (Beukers-Stewart and Jones, 2004) showed a strong preference for clupeids and the avoidance of pomacentrids in the grouper species *Cephalopholis boenak* and *C. cyanostigma* in Australia, and it was concluded that this selective feeding behavior contrasted with the perception of opportunistic predation. The agreement between Beukers-Stewart and Jones's and our findings shows that grouper predation may diverge from opportunistic predation more commonly than previously thought.

The concurrent avoidance of pomacentrids by *C. argus* in Hawaii and Australia indicates a low vulnerability of this family to grouper predation. An explanation may lie in the close reef association and resulting proximity to refuges of many pomacentrids (Beukers-Stewart and Jones, 2004). Incidentally, such a connection between refuges and low vulnerability to predation may help to explain the recent observation by Jones et al. (2004) that coral-associated fishes (including pomacentrids) decline with loss of coral cover during bleaching episodes. At the same time, the few pomacentrids in the diet of *C. argus* in Hawaii belonged to the reef-associated genus *Stegastes*, whereas *Chromis* spp., which are abundant in mid-water, were completely absent. Such low vulnerability of mid-water pomacentrids may be related to the close reef association of *C. argus*, which rarely ventures into the open water column (Hobson, 1974). Low clupeid abundance on Kona reefs in 2003 presumably explains the absence of this family in the diet of *C. argus*.

The terms "preference" and "avoidance" as related to electivity indices do not necessarily solely reflect active choice (e.g., one that is based on nutritional value) but also depend on the vulnerability of prey to capture (Scharf et al., 1998). In the present study, this differentiation was useful in interpreting electivity patterns. In particular, vulnerability provided a straightforward explanation for the contrast between a strong preference for the nocturnal priacanthids and holocentrids and a strong avoidance of the equally nocturnal apogonids: whereas the two preferred taxa hide under ledges or in caves during daytime, which are commonly frequented by *C. argus* (Randall, 2007), the apogonids hide in small reef crevices inaccessible to a large-body predator. Similarly, the complete lack of cryptic families (e.g., Blennidae, Gobidae, Scorpaenidae) and the rareness of planktonic taxa in the diet of *C. argus* may be due to their low vulnerability, if one considers the low visibility of and proximity to cover for cryptic taxa and the usually loose reef association for planktonic taxa.

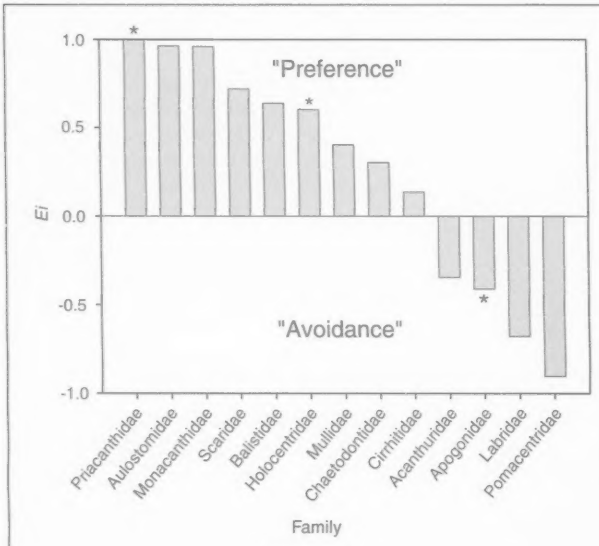

Figure 4

Ivlev's electivity index E_i for prey fishes present in the diet of *Cephalopholis argus* in Kona (Kuhliidae excluded because of a lack of observations in reef fish surveys). Positive values of E_i indicate higher relative importance ("preference") in the diet than in the environment, and negative values of E_i indicate lower relative importance ("avoidance") in the diet than in the environment. Abundances of nocturnally active species (*), underestimated by daytime surveys, were adjusted on the basis of abundances observed in nighttime surveys (see *Methods* section).

The patterns observed for nocturnal taxa, as well as the low importance of crustaceans and large importance of diurnally active fishes in *C. argus* diet, indicate that feeding of this predator in Hawaii was diurnal or crepuscular. This finding confirms that of Hobson (1974) that *C. argus* is a diurnal feeder in Hawaii and is consistent with limited nocturnal movement of *C. argus* in Hawaii (A. Meyer, personal commun.[1]). In contrast, *C. argus* in Madagascar feeds during day and night (Harmelin-Vivien and Bouchon, 1976). The rhythm of feeding activity of *C. argus* thus appears to be variable among regions.

Regarding diurnally active prey taxa, differential vulnerability may offer an explanation for divergent electivity among the abundant families Pomacentridae, Labridae, and Acanthuridae, and among moderately abundant families such as the Chaetodontidae and Scaridae. However, it cannot reasonably account for the strong preference for the rare aulostomids, holocentrids, and monacanthids. This latter pattern appears to be an artifact of recruitment pulses and is further discussed in the context of size selection below.

[1] Meyer, A. 2009. Hawaii Division of Aquatic Resources, 1039 Sand Island Parkway, Honolulu, HI 96821.

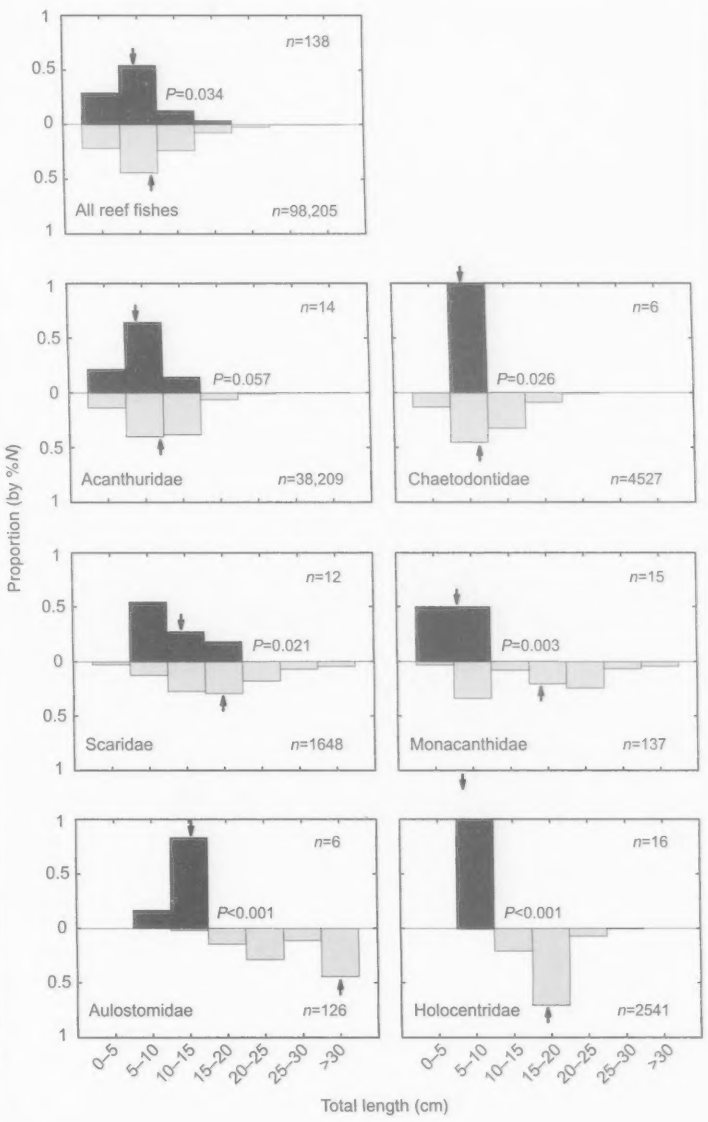

Figure 5

Comparison of the size distribution of prey fishes in the diet of *Cephalopholis argus* (dark gray bars), and of the same prey taxa observed during underwater visual surveys (WHAP surveys) on reefs in Kona (light gray bars), for all fishes combined, and for six of the most important families in the diet of *C. argus* (by %*N*). Sample sizes (*n*) in the upper half of each panel refer to the number of samples of each taxon found in *C. argus* stomachs, and in the lower panel to the total number of individuals of the taxon observed in WHAP surveys in 2003. Mean total lengths for distributions are indicated by arrows. *P*-values are outcomes of Kolmogorov-Smirnov comparisons of the two size distributions in each panel, and significant values indicate that distributions were different.

Size selection

The patterns of prey-size selection by *C. argus* in Hawaii are of particular interest in the context of questions regarding the high mortality of coral reef fish recruits (here defined as postsettlement individuals less than several months of age). During recruitment pulses, large numbers of fish that have completed their planktonic phase settle on reefs (Walsh, 1987). Mortality of these recruits in the first 100 days after arrival can exceed 99% (Werner and Gilliam, 1984; Doherty et al., 2004). Although predation is thought to be an important cause of this mortality (Beets, 1997; Webster, 2002), few studies have conclusively shown the implication of specific predators (Connell, 1998; Beukers-Stewart and Jones, 2004). In this study, the majority of aulostomids, holocentrids, and monacanthids consumed by *C. argus* were recruits, as indicated by comparison of sizes in the diet (TL as small as 9.3, 5.2, 3.8 cm, respectively) with published size ranges for different life history stages of these families (Leis and Carson-Ewart, 2000; Randall, 2007). This finding indicates that the large-body predator *C. argus* contributes to early mortality of reef fishes.

Interestingly, the smallest consumed sizes of the prey families above were rarely or never observed in WHAP underwater visual surveys. This inability to account for recruits may in part be due to low detectability of these small and potentially cryptic individuals. However, the surveys were designed to account for fishes closely associated with the bottom, new recruits, and fishes hiding in cracks (Tissot et al., 2004). It therefore appears more likely that high mortality of recruits between their arrival on the reef and the occurrence of the surveys (4–6 surveys per site in 2003, i.e., every 2–3 months) explains this pattern. Considering the spatial and temporal heterogeneity of recruitment (Dufour et al., 1996), predation on recruits may also account for the divergent dietary importance of small holocentrids and monacanthids between islands in this study.

In contrast to the focus on recruits for the families above, for several families of smaller-body individuals (e.g., Acanthuridae, Chatodontidae), differences between mean size in the diet and in the wild were small. In these cases, comparison with published size ranges (Leis and Carson-Ewart, 2000; Randall, 2007; Claisse et al., 2009) showed that consumed individuals represented in large parts juveniles and small adults, and not recruits. This result indicates that ecological effects of *C. argus* predation differ between prey families. In particular, although predation-induced mortality in several families of larger-body individuals would be limited to recruits (adults finding escape from predation through their large size), in several families of smaller-body individuals, it can also affect juveniles and adults. From a predator perspective, *C. argus* nutrition was then based on 1) accessible sizes of reef fishes such as Acanthuridae, Chaetodontidae, or Scaridae, present year-round on the reef, and 2) recruitment pulses providing access to small individuals of different families, including the Aulostomidae and Monacanthidae, of which adults escape predation because of their large size.

Methodological considerations

Despite the large sample size of the present study compared to that of many previous grouper feeding studies, cumulative prey curves indicated that sample size was too small to fully characterize dietary breadth at the species level, and dietary breadth of Oahu *C. argus* at the family level. This stresses the importance of assessing sample size sufficiency in grouper studies with analytical tools such as cumulative prey curves (Ferry and Cailliet, 1996). The strong correlation of the number of identified taxa with the sample size of *C. argus* studies in the literature impressively confirms the importance of this issue. Secondly, several authors have pointed out that daytime visual surveys may not accurately reflect the importance of nocturnal taxa (e.g., Ackerman and Belwood, 2000). The 90-fold higher abundance of apogonids observed in nighttime surveys compared to that observed in daytime surveys at the same sites in this study underscores this potential limitation of daytime surveys. Finally, in this study, feeding on recruit-sized individuals of some prey fishes of which adults were only rarely observed on Kona reefs was able to explain the high preference for these species. This demonstrates that inclusion of ecological information to interpret electivity values is as important as the choice of a suitable index of electivity (Lechowicz, 1982).

Conclusions

The dominant position of *C. argus* in the guild of large piscivores in Kona shows that this species has become an important component of MHI reef ecosystems since its introduction and raises the question of how this species affects native fishes. The differences in the strength and mechanism of effects of predation among prey families in this study indicate that *C. argus* has the potential to affect the composition of reef fish assemblages. However, structuring effects of predation on fish assemblages are also important in systems without introduced predators (Hixon, 1991). In addition, native predators in the MHI have sharply declined owing to overfishing over the past century—a decline that is reflected in low predator densities in the MHI compared to the remote northwestern Hawaiian Islands (protected from fishing) (Friedlander and DeMartini, 2002). This raises the question of whether *C. argus* is only fulfilling the ecological role previously played by native predators. The results presented here do not suffice to answer these questions. However, by identifying the prey taxa and sizes constituting *C. argus* diet, they open the way partly (the missing part being data on *C. argus* daily ration) for a quantitative estimate of consumption by this species (Bromley, 1994), which could further advance our understanding of its effects on native fishes in Hawaii.

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Electrical phase angle as a new method to measure fish condition

M. Keith Cox (contact author)

Ron Heintz

Email address for contact author: Keith.Cox@noaa.gov

NOAA-National Marine Fisheries Service
Alaska Fisheries Science Center – Auke Bay Laboratories
11305 Glacier Hwy
Juneau, Alaska 99801

Abstract—In this study, phase angle (the ratio of resistance and reactance of tissue to applied electrical current) is presented as a possible new method to measure fish condition. Condition indices for fish have historically been based on simple weight-at-length relationships, or on costly and time-consuming laboratory procedures that measure specific physiological parameters. Phase angle is introduced to combine the simplicity of a quick field-based measurement with the specificity of laboratory analysis by directly measuring extra- and intracellular water distribution within an organism, which is indicative of its condition. Phase angle, which can be measured in the field or laboratory in the time it takes to measure length and weight, was measured in six species of fish at different states (e.g., fed vs. fasted, and postmortem) and under different environmental treatments (wild vs. hatchery, winter vs. spring). Phase angle reflected different states of condition. Phase angles <15° indicated fish in poor condition, and phase angles >15° indicated fish that were in better condition. Phase angle was slightly affected by temperatures (slope=−0.19) in the 0–8°C range and did not change in fish placed on ice for <12 hours. Phase angle also decreased over time in postmortem fish because of cell membrane degradation and subsequent water movement from intra- to extracellular (interstitial) spaces. Phase angle also reflected condition of specific anatomical locations within the fish.

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For nearly a century, fisheries biologists have struggled to develop a way to simply and accurately assess body composition and condition of fish (Adams et al., 1993; Shearer et al., 1994). Attempts at assessing body composition or creating a condition index have focused on simple relationships between length and weight of fish (Le Cren, 1951; Anderson and Neumann, 1996). These early methods were later replaced by formulations of length and weight information such as relative weight (*Wr*) by Wege and Anderson (1978) and Fulton's condition factor by Murphy et al. (1990), which are easily obtained, but lack sensitivity specific to an individual's body composition. In contrast, more difficult and technical approaches involving necropsy, histology, or pathology (NHP) can provide more detail, but these approaches can not conducted easily in the field (Strange, 1996). The more technical approaches of NHP can provide detailed information about individual fish, but the cost and technical expertise required to conduct them has restricted field biologists' ability to effectively apply these methods on broad scales.

A bridge is needed between simple, cost-effective, and robust length-weight regressions and complex, expensive, yet sensitive laboratory methods. Recently, common ground has been found by Cox and Hartman (2005) in body composition estimates with the use of bioelectrical impedance analysis (BIA). This method still depends on chemical analysis of subsets of fish in order to develop calibration curves that relate resistance (R) and reactance (Xc) to body composition, but analytical costs are reduced because after a curve is created, there is no longer a need for body composition analysis. Use of BIA relies on correlations between the electrical conductivity of fish tissues and body composition. Thus, BIA is an indirect measure of total body water (*TBW*), dry weight (*DW*), fat-free mass (*FFM*), total body protein (*TBP*), total body ash (*TBA*), total body fat (*TBF*), or mass-specific energy density (*ED*). Another BIA method currently used in human health studies involves the phase angle (ratio of Xc and R) as a direct measure of nutritional condition (Barbosa-Silva and Barros, 2005). In these studies, phase angle indicates cell membrane potential and water distribution between the intra- and extracellular spaces and is used widely in human medicine as a means to measure nutritional status, but it has never been applied to fish or lower vertebrates.

Phase angle represents the relationship between the two vector components R and Xc that represent impedance. Specifically, phase angle is defined as

$$\textit{Phase angle} = \arctan(X_c / R)180°/ \Pi, \qquad (1)$$

where R and Xc are measured in ohms.

Phase angle ranges between 0° and 90°; 0° if the circuit is only resistive (as in a system with no or degraded cell membranes), and 90º if the circuit is only capacitive (all membranes have no extracellular fluid). In either fish or human health studies, 45° phase

angles reflect circuits that consist of equal amounts of capacitive reactance and resistance (Meeuwsen et al., 2001). Lower phase angles appear to be consistent with low reactance and either cell death or a breakdown in the selective permeability of the cell membrane (Schwenk et al., 2000a, 2000b). Higher phase angles are consistent with high reactance and large quantities of intact cell membranes and body cell mass (Abu Khaled et al., 1988; Foster and Lukaski, 1996). The use of phase angle as a human health indicator is becoming more common in medical fields (Dejmek and Miyawaki, 2002; Damez et al., 2007). Even when other anthropometric evaluation methods such as body mass index (BMI) and skin-fold tests are not accurate or sensitive to tissue change, phase angle has been found to be accurate. Furthermore, phase angle is considered to be a possible global marker of health and evaluation of cell membrane function, and consequently serves as a prognostic tool for human disease (Barbosa-Silva and Barros, 2005).

We demonstrate that phase angle can be used to measure the nutritional condition of fish under laboratory and field conditions. Our objective was to determine whether fish known to have decreasing nutritional status have lower phase angles than those fish with high nutritional status. We made these measurements on six species of fish: brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*O. tshawytscha*), chum salmon (*O. keta*), pink salmon (*O. gorbuscha*), and Pacific herring (*Clupea pallasii*) under laboratory conditions and on fish collected from the field. In addition, we examined postmortem changes in phase angle in fish to illustrate how phase angle reflects membrane integrity and water balance.

Materials and methods

We tested the hypothesis that phase angle changes with the nutritional status of fish by conducting three laboratory experiments and three field studies using live or freshly killed fish. Throughout this study, we assume that malnourished, over-wintering, and migrating anadromous fish are in diminished nutritional condition compared to their nourished, prewinter, and premigratory counterparts. This assumption has been thoroughly evaluated in the literature where condition declines have been found to be synonymous with depletion of stored fat, protein, and carbohydrates (Adams et al., 1982; Weatherup and McCracken, 1999). We also monitored phase angle in postmortem adult salmon to measure how phase angle changes in response to cell degradation and water movements from intra- to extracellular spaces. In all cases, phase angles were calculated from impedance measures (R and Xc) of fish that were sampled according to the methods in Cox and Hartman (2005). In this study, two sets of needle electrodes (stainless 28 gauge, Grass Telefactor, West Warwick, R.I.) each consisting of a signal and detecting electrode were inserted to a depth of 1 cm. One set was placed towards the caudle peduncle and the second set was placed in the nape region of the fish. Variables that could introduce error in R and Xc measures (e.g. varying the depth and gauge of needles, placement of fish on different conductive surfaces) were standardized to negate any bias, whereas temperature and time effects are explained experimentally.

Temperature and time

To test for significant effects of temperature on impedance measurements from dead fish, regression analysis was used to test whether slopes and intercepts differed from zero on regressions of temperature and response measures (phase angles). Three adult pink salmon (520–550 mm fork length) were killed and connected to a BIA Quantum-II Desktop System (RJL Systems, Point Heron, MI) using standard needle electrodes and orientations as described by Cox and Hartman (2005). The Quantum-II was set to record impedance every five minutes for 12 hours. An ibutton thermometer (Maxim Integrated Products Inc., Sunnyvale, CA) was placed 3 cm inside the dorsal musculature of the fish and was set to record temperatures every 5 minutes. Both impedance measurements and thermometers were synchronized before the experiment. Each fish was brought directly from the water, killed, and placed on a 4-inch wire stand (to allow air flow around the fish) in the empty freezer compartment of a standard freezer. Initial fish temperatures were equal to ambient water temperature of 8°C. After 12 hours, the fish was removed from the freezer and impedance measures and temperature data were downloaded onto a computer. For regression analysis, only impedance measures taken when the fish temperature was between 0°C and 8°C were used. Impedance measures of R and Xc were used to calculate phase angle measures. Significance tests to test for nonzero slopes were done on each fish by using a standardized major axis (SMA) test and between fish by using the Bartlett-corrected likelihood ratio (LR) test for differences in the slopes. An analysis of covariance was used to test effects of temperature and individual fish on phase angle.

We examined the effect of time after death on response measures (phase angles) to determine time effects on phase angle. Juvenile coho salmon (n=30, mean=10 g, standard deviation [SD]=2.72) from the Sheldon Jackson Hatchery, Sitka, AK, were killed and groups of six fish were randomly placed in plastic bags and placed on ice. At 0-, 3-, 6-, 9-, and 12-hour intervals, a bag was removed and six fish were measured for length, weight, and impedance. Impedance measures followed standardized procedures found in Cox and Hartman (2005). Impedance measures were then used to calculate phase angle. Linear-effects mixed models were used to test for effects of time on phase angles.

Laboratory study 1: fasted and fed brook trout

To determine the effects of malnutrition on phase angle in brook trout in fresh water, phase angle was repeatedly measured in fed and fasted juvenile brook trout over a period of nine weeks (December 2002–March 2003).

We obtained brook trout (n=16, mean weight=15.29 g, SD=1.21) from the Bowden State Fish Hatchery, Bowden, WV, and transferred them to West Virginia University. Before transfer, fish were fed standard hatchery trout pellets *ad libitum* approximately three times per day. After transfer, fish were randomly selected to be in one of two groups (n=8): fasted or fed. Each fish was housed in individual 33L aquaria at 14–16°C. Fish in the fed group were offered *ad libitum* rations of fly larvae (*Sarcophaga bullata*) (Grubco Inc., Hamilton, OH). Each week for nine weeks, all fish were anesthetized with a solution of 1 g tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Inc., Redmond, WA) to 9 L water, and measured for length, weight, and impedance (i.e., eight replicates for each of two groups every week for nine weeks) according to the methods in Cox and Hartman (2005).

Laboratory study 2: fasted and fed rainbow trout

The effect of malnutrition on phase angle measures was measured in rainbow trout in fresh water by repeatedly measuring the phase angle in fed and fasted juvenile rainbow trout over a period of four weeks starting in December 2004. Rainbow trout used in this study were raised and housed at the U.S. Department of Agriculture, Agricultural Research Service, National Center for Cool and Cold Water Aquaculture (USAD/ARC NCCWA) in Leetown, WV. Two weeks prior to the experiment, 11 small (mean weight=68.76 g, SD=6.02) and 12 large (mean weight=193.03 g, SD=15.72) fish were individually placed in 2.75-L and 9.0-L aquaria and fed standard hatchery pellets *ad libitum*. Two days before the start of the experiment, all fish were fed standard hatchery pellets at a rate of 2% body weight per day. At the start of the experiment each size class of fish was randomly split into two groups: fasted (n=11) or fed (n=12). Fed fish were offered hatchery pellets at a rate of 2% body weight per day and all holding tanks were kept at 15°C for the duration of the experiment (four weeks). Every week for four weeks, each fish was anaesthetized with MS-222 (1g/9L water) and measured for impedance.

Laboratory study 3: fasted and fed Chinook salmon

We tested the effect of malnutrition on phase angle measures in Chinook salmon in salt water. Impedance was repeatedly measured in fed and fasted juvenile Chinook salmon over a period of 13 weeks (mean weight=10.25 g, SD=2.23). In November 2007, fish reared at the National Marine Fisheries Service (NMFS) research hatchery at Little Port Walter, AK, were transferred to the Auke Bay Laboratories, Juneau, AK. Fish were acclimated for approximately one month during which time salinity was increased from 0 to 32 and were then randomly assigned to fasted (n=7) or fed (n=12) groups. Pairs of individually marked fish (fin clipped) were placed into 8-L aquaria and held at 4°C. Bioelectrical impedance was measured in individual fish on weeks 1, 8, 11, and 13, and different numbers of fasted and fed fish were measured each week. The number of fed and fasted fish measured in each sampling week were as follows: week 1 (n=12 and 7); week 8 (n=4 and 7); week 11 (n=8 and 5); and week 13 (n=5 and 5).

Differences in phase angle in the laboratory studies were analyzed by a repeated measures analysis of variance (ANOVA). The analysis was used to test for 1) significance differences in phase angle means between fed and fasted groups, 2) a time effect on group means, and 3) interactions between time and group means. A linear mixed-effects (LME) model for repeated measures was used to compare phase angles from the start of the experiment with those measured after the start.

Field study 1: wild and hatchery brook trout

To determine if phase angles differed between hatchery and wild fish, phase angles were compared between wild fish foraging under natural conditions and captive fish fed *ad libitum*. In July 2002, phase angle was measured in 56 brook trout, 34 from headwater streams (mean weight=21.21 g, SD=12.85) and in 11 brook trout from Bowden State Fish Hatchery, Bowden, WV (mean weight=43.17 g, SD=4.76). Before they were measured, hatchery-reared trout were fed standard trout pellets *ad libitum* three times each day according to standard hatchery procedures. Wild fish were captured by electrofishing from unidentified streams located within the Middle Fork watershed in Randolph County, WV. After capture, impedance was immediately measured in both groups. A two-sample t-test was used to compare calculated phase angles between hatchery and wild fish. Internal fish temperature was not measured, but water temperatures measured in both areas were similar (mean=13.36°C, SD=0.94).

Field study 2: phase angle in migrating chum salmon

The effect of migration on phase angle was compared in migrating adult chum salmon. On 4 August 2004, a total of 47 chum salmon (mean=3438.29 g, SD=511.41) were collected by gillnet from a downstream site in the Yukon River near Emmonak, AK. During 27–29 September 2004, 40 chum salmon (mean weight=2927.50 g, SD=470.13) from an upstream site in the Yukon River near Delta, AK (approximately 3200 km upstream from Emmonak), were collected by gillnet. Impedance measures on both the dorsal and ventral (gonadal) areas of each fish were measured within one hour of capture. Internal temperatures were taken by a digital thermometer on both groups of fish in the anal vent (downstream mean temperature=16.44°C, SD=1.22; and upstream mean temperature=3.00°C, SD=0.48). A two-sample t-test was used to compare phase angles of upstream and downstream salmon.

Field study 3: overwintering Pacific herring

We monitored phase angle in Pacific herring during winter to determine if decreases in fish condition due

to season could be detected. During the winter of 2006, Pacific herring were sampled in January (n=68, mean weight=111.26 g, SD=41.00), February (n=70, mean weight=8.98 g, SD=1.86), March (n=68, mean weight=35.92 g, SD=56.37), and April (n=23, mean weight=166.14 g, SD=36.30) from Sitka Sound, AK. Fish were collected by cast net. Impedance was measured within 12 hours of capture and fish were held on ice or snow between capture and measuring. After measuring, a subset of the fish (January n=20, March n=14, April n=18) was sent to the Auke Bay Laboratory, Juneau, AK, for bomb calorimetry. A linear mixed-effects (LME) model was used to test for differences in phase angles between months.

Phase angle and water balance in postmortem fish

Adult pink salmon were measured to characterize changes in phase angle in postmortem fish to better understand how changes in cell integrity influence phase angle. Three adult pink salmon (520 mm–550 mm) were individually killed and connected to the Quantum-II and placed in a standard ice chest without ice and held at <11°C. The Quantum-II was set to record impedance every five minutes for five days. A temperature data logger placed inside the ice chest recorded temperature every five minutes. Each fish was removed from the ice chest 4–5 days later and impedance measures and temperature data were downloaded for analysis. Changes in phase angle of postmortem fish over time were analyzed by regression analysis. Significance tests were done on each fish to test for nonzero slopes by using a standardized major axis (SMA) test and by reporting the Bartlett-corrected likelihood ratio test (LR) for differences in the slopes between the different fish.

Results

Temperature and time

Phase angles measured on dead fish depended on temperature and decreased as temperatures increased (Fig. 1A). Slopes of phase angles (–0.19) were not different among fish (LR=5.87, P=0.05). There was an interaction between fish and phase angle ($F_{2, 46}$= 20.92, P=0.01). Time did not affect phase angles in fish that were placed on ice for up to 12 hours. Phase angles measured in groups of dead fish placed on ice were not significantly different between times of less than 12 hours, (LME $t_{30, 30}$<2, P>0.08) (Fig. 1B). At 12 hours, there was a significant effect of time on phase angle (LME $t_{30, 30}$>5, P<0.05).

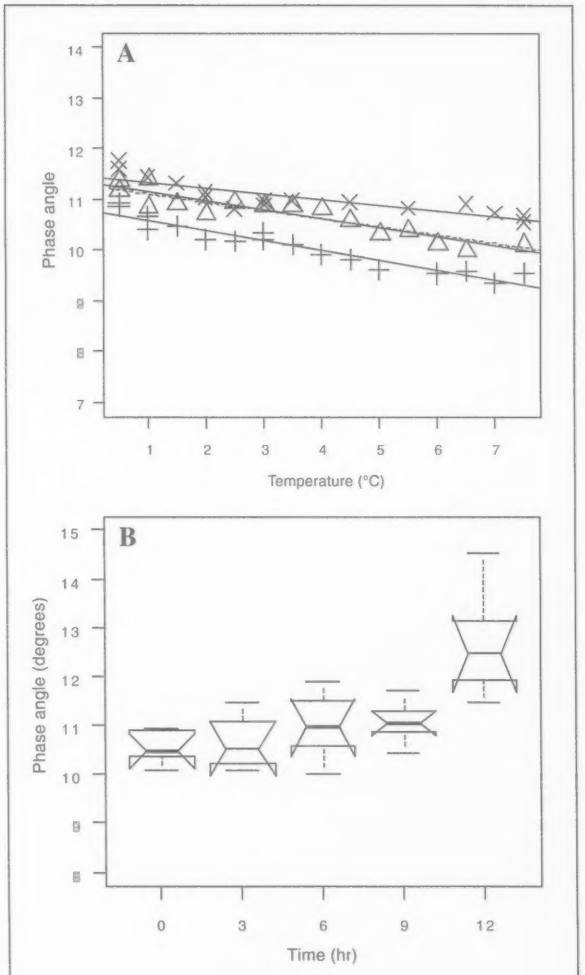

Figure 1

(**A**) Repeated measures of phase angles for three adult pink salmon (*Oncorhynchus gorbuscha*) that were brought from 8°C to freezing. Time required for freezing was <1 hour. Each of the three symbols and their corresponding line represents one of the three fish. (**B**) Phase angles of group means for juvenile coho salmon (n=6 per group) that were killed and placed on ice. Every 3 hours, a group was measured for impedance, and the phase angle means per group were calculated. Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals, horns indicate that the interval is larger than the interquartile range (as seen in the bottom half of each of the boxplots).

Laboratory studies 1–3

In each of the laboratory studies, phase angle decreased in fasted fish and not in fed fish. In the brook trout experiment there was a significant interaction in phase angle between feeding group and time (ANOVA, P<0.05, df=8). The interaction resulted in a temporal change in phase angle among the fasted fish—a change that was not observed among fed fish. Phase angles in the fasted brook trout in weeks 3–9 were significantly lower than

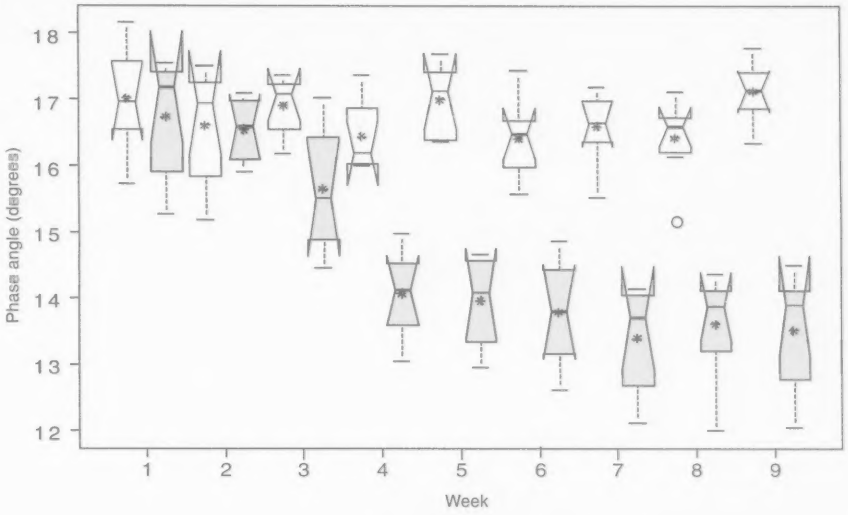

Figure 2

Notched boxplots and means (*) of phase angles measured weekly for nine weeks from brook trout (*Salvelinus fontinalis*) that were fed ad libitum (white boxes, n=8) or fasted (gray boxes, n=8). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals; horns indicate that the interval is larger than the interquartile range. Open circle (○) represents outlier determined by a Grubbs test.

those in week 1 (LME, P<0.001, df=63). In contrast, there were no differences in phase angle between weeks 2 and 9 and week 1 in the fed fish (LME, P>0.05, df=63) (Fig. 2). The fed group increased in size (mean=15.5 g to 68.5 g) over the same time period, whereas the fasted group lost mass (mean=15.0 g to 11.9 g) (Cox, 2004).

In rainbow trout, ANOVA results were similar for both small and larger fish and interactions of phase angle between feeding group and time were significant (ANOVA, P<0.05, df=3) (Fig. 3, A and B). In the fasted group for both small and large fish, phase angle was significantly lower in weeks 3 and 4 than in week 1 (LME, P<0.03, df=40). At the start of the experiment one fish from the fed group died; however, no changes in phase angle were detected between weeks 2–4 and week 1 in the fed group (LME, P>0.09, df=43). Small fish in the fed group grew in size from a mean of 66.4 g to 98.7 g, and large fed fish grew from an average of 197.2 g to 297.7 g. Likewise, small fish in the fasted group lost weight from a mean of 87.8 g to 80.5 g, and larger fasted fish decreased in weight from an average of 188.7 g to 172.0 g.

Results of the Chinook salmon experiment were similar to those of the brook and rainbow trout experiments, but phase angles in the saltwater fish were generally lower than those of the freshwater fish. The interaction between feeding group and time was significant (ANOVA, P<0.001, df=3) as phase angle decreased with time in the fasted group (LME, P<0.009, df=20), whereas it increased in the fed group (LME, P<0.04, df=20) (Fig. 4). Phase angle changes were consistent with changes in wet mass. The fasted fish lost an average 0.2% of their wet mass per day. In contrast, the fed group gained an average 0.2% of their wet mass per day. Phase angles were lower in Chinook salmon than in rainbow or brook trout in both fed and fasted groups. Phase angles for Chinook salmon and rainbow, and brook trout in the fed groups averaged 15.6°, 16.2°, and 16.5°, respectively, and 13.4°, 15.0°, and 14.5°, respectively, for the fasted groups.

Field studies 1–3

Phase angle reflected changes in the presumed nutritional status of wild fish in each of the field studies. In comparisons between hatchery and wild trout, mean phase angle was significantly higher in the hatchery trout (two-sample t, P=0.04, t=2.03, df=53). Mean values for hatchery and wild brook trout were 19.43° and 18.27°, respectively (Fig. 5). Similarly, comparison of adult chum salmon sampled in the Yukon River indicated that upstream fish had significantly lower phase angles (two-sample t, P<0.001, t=16.5, df=72) from the dorsal measures, but not from the ventral measures (two-sample t, P=0.15, t=–1.4, df=65). Downstream and upstream phase angles averaged 23.98° and 17.53°, respectively,

for dorsal measures, and 10.30° and 11.15°, respectively, for ventral measures (Fig. 6). Phase angle also decreased during winter months in Pacific herring collected from Sitka Sound (LME, $P<0.003$, df=225). Phase angle decreased from 12° to 10° from January to March (Fig. 7), whereas mass-specific energy content declined from 7.15 kJ/g to 4.79 kJ/g. Phase angle increased to 15° in April, and mass specific energy increased to 5.02 kJ/g.

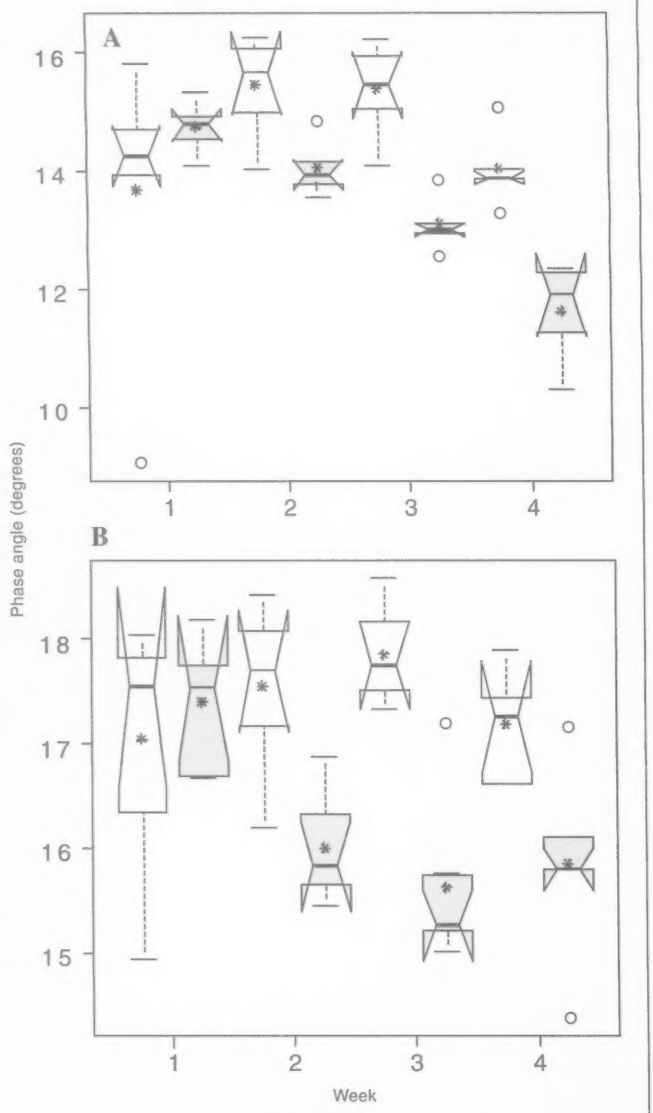

Figure 3

Notched boxplots and means (*) of phase angles measured weekly for 4 weeks from (**A**) small (<100 mm) and (**B**) large (100 mm) rainbow trout (*Oncorhynchus mykiss*) that were fed *ad libitum* (white boxes, n=12) or fasted (gray boxes, n=11). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

Phase angle and water balance in postmortem fish

Phase angles over time in postmortem fish reflected changes in cell integrity and subsequent water movement from intra- to extracellular spaces, reflected by the two components (R and Xc) which are used to calculate phase angle. In all three dead fish, slopes were initially positive reflecting an increase in Xc and a concomitant increase in R. Within 12 hours of death, this process reversed and slopes became negative as phase angle decreased (Fig. 8). There was not enough evidence to indicate that slopes were different between fish (LR [likelihood ratio]=110.7, $P<0.001$, slope=−4.2).

Discussion

In this study, in both laboratory and field settings, phase angle was used to compare the relative nutritional condition of salmonids and clupeids in fresh and saltwater while each was in a different condition. In each case where fish were expected to be in poor condition, phase angle was lower than in fish in good condition. The range of phase angle values was also greater in the larger rainbow trout than in the smaller rainbow trout. Phase angle reflects changes in condition by directly measuring the R and Xc of the body tissue, and more specifically, the ratio of these two values directly represents changes in intra- and extracellular water distributions (i.e., intracellular dehydration and extracellular hydration) (Schwenk et al., 2000b). Water distribution (namely, the movement from intra- to extracellular spaces) can be attributed to use of energy stores as indicated by the herring study in field study 3. In humans, phase angle can reflect loss of body protein during starvation as well as presence of infection, both of which decrease the condition of the organism (Plank et al., 1998; Schwenk et al., 2000a). Changes in phase angle in fish are therefore likely to reflect the general health of fish in addition to their nutritional status. Consequently, phase angle should be considered a reliable independent marker of fish condition.

Phase angle changes with nutrient levels in fish. When fish fast, nutrient inputs of

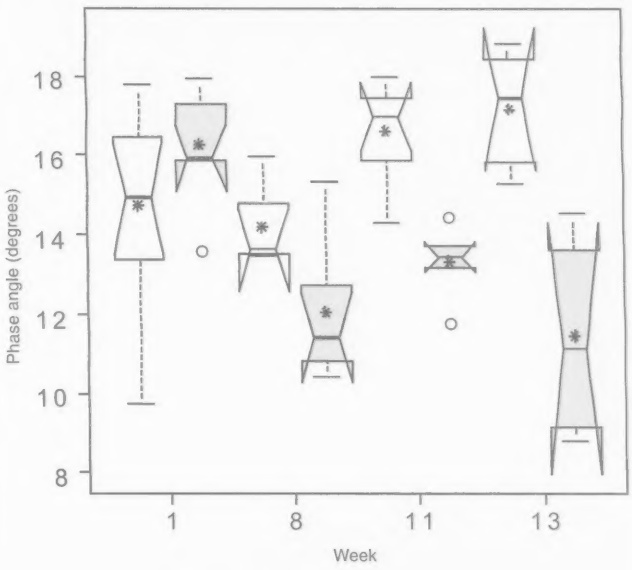

Figure 4

Notched boxplots and means (*) of phase angles measured during weeks 1, 8, 11, and 13 from Chinook salmon (*Oncorhynchus tshawytscha*) that were fed *ad libitum* (white boxes, n=12) or fasted (gray boxes, n=7). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

proteins, carbohydrates, and fats are lacking, which forces the use of stored nutrients to meet energetic demands (Moyle and Cech, 2004). Consistent with this use of stored nutrients are shifts in intra- and extracellular water, and findings by Finn et al. (1996) show that loss of body protein parallel a loss of intracellular water causing subsequent cell shrinkage and progressive cellular dehydration. As fish fasted, cells likely became more and more dehydrated and phase angles decreased. This has also been observed in humans with anorexia nervosa, where low phase angles reflect decreased nourishment (Mika et al., 2004). As organisms continue to starve, phase angles continue to decrease as stored cofactors such as vitamins, which are needed for metabolic conversions, are depleted and cause a further decline in condition. Typical symptoms of vitamin deficiencies include muscle and cellular atrophy, poor growth, and anemia, all of which would lower phase angles. As phase angle changes with the nutritional status of laboratory-fasted fish, it is apparent that phase angle can be used to determine if a fish has been subjected to a food-limited scenario.

When we compared hatchery fish to wild fish, we found that phase angle was lower in the wild fish, indicating that the condition of wild fish was lower. It can be assumed that food is not a limiting factor in hatchery fish, nor is there a need to forage. The opposite is true in wild fish, where food is usually limited and variable, and where there is almost always a need to forage. In the optimal foraging theory it is assumed that when food is limited, all energetic functions are not fulfilled and energy must be allocated to different different physiological parameters to maximize survival of the animal (Molles, 2005). This conclusion is supported by Berg and Bremset (1998) who found that there are seasonal changes in the body composition of juvenile salmonids that are due to changing energy allocations. In hatchery fish, foraging costs are reduced, risk of predation is minimized, and food is abundant. Furthermore, the weights and condition of wild foraging fish would naturally be more variable (as seen in these data) because both of these parameters are dictated by numerous variables. Any excess energy consumed by hatchery fish is allocated towards growth and storage and concurrently provides the fish with proper vitamins and minerals to maximize growth. Phase angle was lower in wild fish where they may have had to use energy for foraging and storage.

The lower phase angles observed in upstream adult salmon were due to their presumed diminished condition resulting from consumption of endogenous energy stores and to increased extracellular water volume. Like

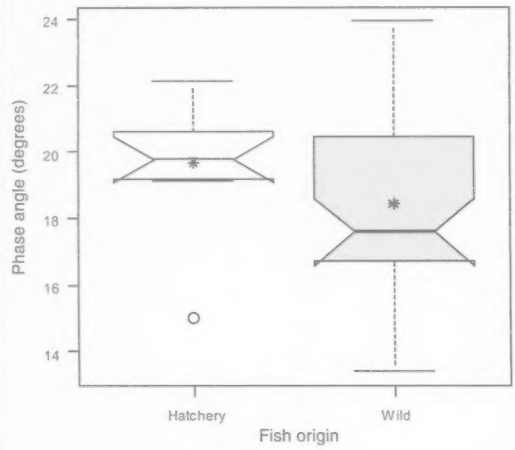

Figure 5

Notched boxplots of phase angles and means (*) from brook trout (*Salvelinus fontinalis*) from a hatchery (n=21) and from the wild (n=35). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circle (○) represents outlier determined by a Grubbs test.

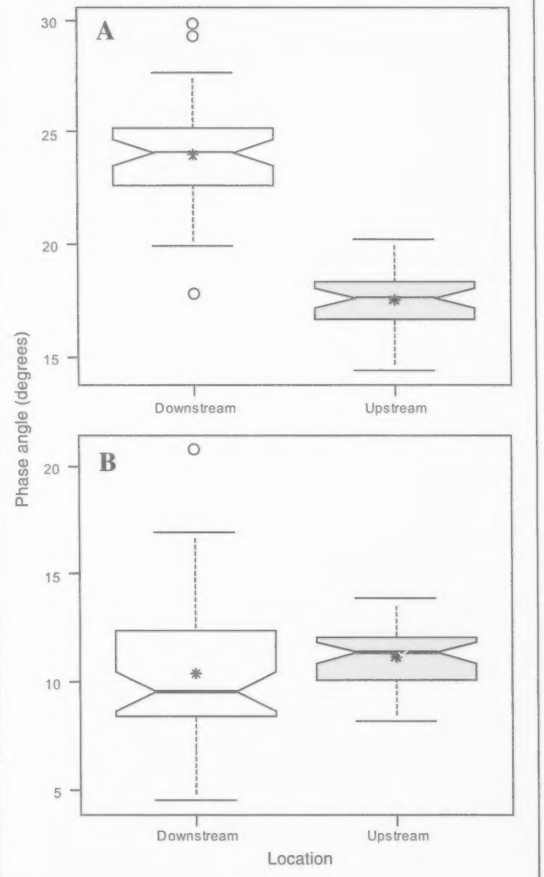

Figure 6

Notched boxplots of phase angles and means (*) calculated from bioelectrical impedance analysis measurements taken on the dorsal (**A**) and ventral (**B**) sides of chum salmon (*Oncorhynchus keta*) at both the mouth of the Yukon (downstream) (n=47) and 2000 km upstream (n=40). Notches extend to ±1.58 interquartile range/$\sqrt{n}$ and represent roughly 95% confidence intervals. Open circles (○) represent outliers determined by a Grubbs test.

the fasted fish in the laboratory studies, anadromous salmon fast as they migrate upstream to spawn and must rely on stored fat and protein as energy sources during their journey. Jonsson et al. (1997) found that during upstream migrations, percent somatic and visceral lipid content can decrease from 12% to 2%, and 11% to 1%, respectively, and total stored energy losses can total 60%. Both cellular degradation and extracellular hydration would result in decreasing phase angles from the dorsal tissue.

Lower phase angles were observed in the ventral musculature, confirming that phase angle measures are location-specific. Phase angles calculated from ventral tissue revealed that ventral tissues do not degrade at the same rate as dorsal musculature. Conservation of the cell integrity in the musculature surrounding the gonads is consistent with observations that gonad quality is conserved during migration. Jonsson et al. (1997) found that although migrating salmon experience a marked decrease in energy content of somatic and visceral tissues during upstream migration and spawning, energy content in ventral gonadal tissue remained high throughout migration. It is noteworthy that phase angle was sensitive enough to indicate tissue-specific differences in condition and therefore could have potential use in quantifying reproductive readiness.

The sensitivity of phase angle to nutritional status was further indicated among Pacific herring between March and April. Whereas mass-specific energy content increased by approximately 5% between March and April, phase angle increased by 50%. Multiple studies on Pacific herring indicate reductions in energy and lipid content, and growth rates during over-wintering (Arrhenius and Hansson, 1996; Pangle et al., 2004). As energy stores become diminished from lack of food, physiological condition of the fish declines in a manner similar to the aforementioned "starving" fish. Vollenweider (2005) found that diminished mass-specific energy content in Pacific herring rebounded after spring algal blooms. This rebound occurs after spawning and likely reflects the reallocation of energy from gonad maturation to somatic growth and the replenishment of energy

stores. However, changes in mass-specific energy content can only be detected in tissues when there has been a sufficient change in the amount of lipid in relation to protein. In contrast, phase angle apparently changes much faster, reflecting local alterations in membrane potential and water balance.

Measuring changing phase angles in dead fish provides a useful model for illustrating BIA performance in live fish. Phase angle changes with time after death owing to rigor mortis, cellular breakdown, and water movement from intra- to extracellular spaces. Although timeframes for the onset and resolution of rigor mortis depends on species, condition of fish at time of death, environmental stress, and temperature (Martinsen et al., 2000; Damez et al., 2007), rigor mortis usually occurs within the first 18 hours of death, when muscles remain contracted until resolution. This period is reflected in our data as an increase in phase angle with time. Human studies involving muscle contractions and impedance indicate that muscle contractions result in an increase in R and Xc (Kashuri et al., 2007). Our study was consistent with Kashuri et al. (2007) in that Xc increased during rigor mortis (contraction). Their study indicated that increases in Xc were due to cell membrane and intracellular changes (possibly metabolites) and not to volumetric or morphological changes. In our study, decreases in impedance upon resolution (relaxation) were most likely due to the physical breakdown of cell membranes and subsequent autolysis and nucleotide catabolism. The breakdown of cell membranes and muscle hydrolysis causes the release of electrolytes and water into extracellular space, therefore decreasing the phase angle (area of decreasing slope in Fig. 8). In a study of postmortem changes in dielectric properties of haddock (*Melanogrammus aeglefinus*) muscle, Martinsen et al. (2000) found that the onset and resolution of rigor mortis affected R levels at multiple frequencies and they associated the increase with a response to edema. This makes the use of phase angle applicable to measuring changes during the postmortem period in fish after the resolution of rigor mortis, therefore allowing phase angle to determine time of death in fish where ambient temperature is known.

It is important to know that phase angles were only slightly affected by temperature and not affected by time as long as the fish were placed on ice and measured within 12 hours of capture. Slopes were −0.19, or approximately a 1° drop in phase angle, for every 5°C increase in temperature. There was no effect of time on phase angle on fish iced for less than 12 hours. At about 12 hours, juvenile coho salmon enter rigor mortis and phase angle measures begin to increase. These are important findings because fishery biologists using BIA can minimize error caused by temperature effects by icing fish or by trying to keep temperature fluctuations to a minimum before BIA measurements.

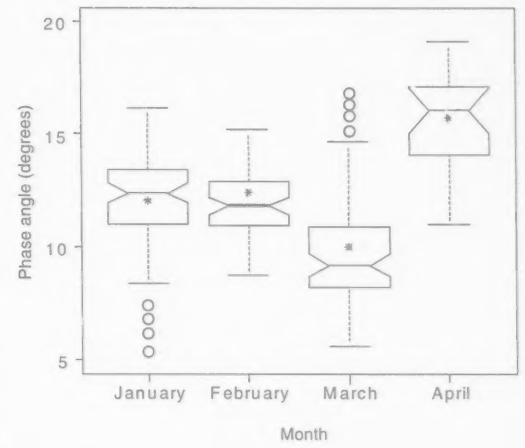

Figure 7

Notched boxplots of phase angle means (*) for Pacific herring (*Clupea pallasii*) (n=229) captured during January, February, March, and April of 2007 in Sitka Sound, Alaska. Open circles (○) represent outliers determined by a Grubbs test.

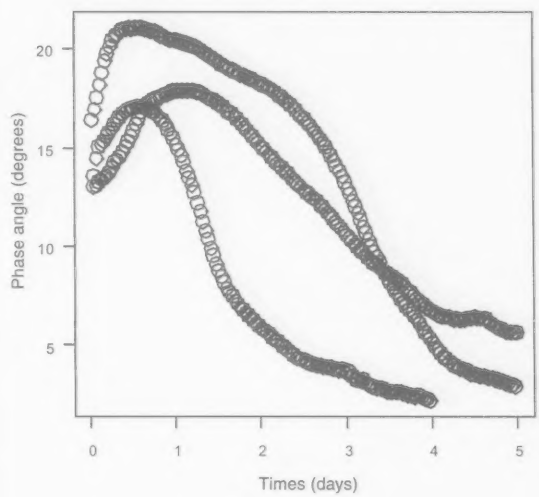

Figure 8

Phase angles for postmortem adult pink salmon (*Oncoryhnchus gorbuscha*) (n=3) measured every 10 minutes for 5 days while stored at temperatures <11°C.

In summary, phase angle reflected the nutritional status of six species of fish in fresh and saltwater after three to four weeks of starvation and reflected the presumed nutritional status of field-caught fish. Re-

gardless of the environment, phase angles decreased with impaired fish condition. The use of phase angle as a direct measure of nutritional status and possibly general health can bridge the gap between indirect nonspecific measures such as length-weight indices and direct specific measures of physiological parameters that are obtained through laboratory analysis. Furthermore, phase angle allows real-time direct measurements of condition in fish in the field without the need to sacrifice them. The low cost and rudimentary technical expertise required to conduct phase angle measurements will allow field biologists and technicians to effectively measure the condition of fish and apply these measurements on broad ecological scales. Although variables were controlled as much as possible in this study, further research should be undertaken to investigate additional potential sources of error that may affect these measures and should include temperature and time of measurement after death as well as the configuration, gauge, and depth of needles used during measurements.

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Abstract—We examined whether the relationship between climate and salmon production was linked through the effect of climate on the growth of sockeye salmon (*Oncorhynchus nerka*) at sea. Smolt length and juvenile, immature, and maturing growth rates were estimated from increments on scales of adult sockeye salmon that returned to the Karluk River and Lake system on Kodiak Island, Alaska, over 77 years, 1924–2000. Survival was higher during the warm climate regimes and lower during the cool regime. Growth was not correlated with survival, as estimated from the residuals of the Ricker stock-recruitment model. Juvenile growth was correlated with an atmospheric forcing index and immature growth was correlated with the amount of coastal precipitation, but the magnitude of winter and spring coastal downwelling in the Gulf of Alaska and the Pacific Northwest atmospheric patterns that influence the directional bifurcation of the Pacific Current were not related to the growth of Karluk sockeye salmon. However, indices of sea surface temperature, coastal precipitation, and atmospheric circulation in the eastern North Pacific were correlated with the survival of Karluk sockeye salmon. Winter and spring precipitation and atmospheric circulation are possible processes linking survival to climate variation in Karluk sockeye salmon.

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Growth and survival of sockeye salmon (*Oncorhynchus nerka*) from Karluk Lake and River, Alaska, in relation to climatic and oceanic regimes and indices, 1922–2000

Ellen C. Martinson (contact author) [1]
John H. Helle[1]
Dennis L. Scarnecchia[2]
Houston H. Stokes[3]

Email address for contact author: Ellen.Martinson@noaa.gov

[1] Auke Bay Laboratories
Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
Ted Stevens Marine Research Institute
17109 Point Lena Loop Road
Juneau, Alaska 99801-8626

[2] Department of Fish and Wildlife Resources
University of Idaho
P.O. Box 441136
Moscow, Idaho 83844-1136

[3] University of Illinois at Chicago
601 S. Morgan Street
Chicago, Illinois 60607

Fish stocks and fisheries in the Gulf of Alaska are strongly influenced by climatic and oceanic (C-O) conditions (Francis and Hare, 1994; Hare and Francis, 1995). In the past century C-O conditions in the North Pacific Ocean and Gulf of Alaska (GOA) have shifted from a warm regime and higher salmon (*Oncorhynchus* spp.) production (1927–46) to a cool regime and low salmon production (1947–76) and back to a warm regime and higher salmon production (1977–2000) (Francis and Hare, 1994; Hare and Francis, 1995).

Changes in C-O conditions, either annually or over longer regime periods, may affect salmon smolt-to-adult survival rates by affecting the growth rate of smolts after they enter the ocean. For example, higher survival rates of Alaska sockeye (*O. nerka*), pink (*O. gorbuscha*), and chum (*O. keta*) salmon have been associated with warmer coastal sea-surface temperatures (SST) during the first year that young salmon spend in the ocean (Mueter et al., 2002a). Relationships have been found between faster early marine growth and higher survival in chum salmon (Healey, 1982), pink salmon (Moss et al., 2005), and coho salmon (*O. kisutch*) (Beamish and Mahnken, 2001). The influence of C-O indices and regime shifts in the GOA on the survival of south central Alaska sockeye salmon has yet to be specifically linked to changes in marine growth rates of fish.

If marine growth influences sockeye salmon survival, then relating marine growth to specific C-O indices over an extended time period would provide insight into environmental factors leading to variation in year-class strength. Although direct information on fish growth (e.g., growth rates of smolts and postsmolts at standard times and locations) is not available, measurements from archived scales can be used to indirectly estimate the freshwater and marine growth rates of age 2.2[1] sockeye salmon that

[1] In expressing age of salmon in this article, numbers before the decimal refer to numbers of freshwater annuli, numbers after the decimal refer to numbers of marine annuli (Koo, 1962).

returned to Karluk River and Lake system on Kodiak Island, Alaska, from 1922 to 2000, with the exception of seven years for which data were missing. Hereafter, we refer to these fish as Karluk sockeye salmon. The objectives of this study were 1) to describe how mean freshwater and marine growth rates of Karluk sockeye salmon varied over multiyear periods in relation to warm and cold C-O regimes in the North Pacific Ocean; 2) to describe how marine growth rates varied annually in relation to annual variations in regional C-O indices; and 3) to describe the interrelationships among annual growth rates, annual C-O indices, and annual survival.

Historically, the production of sockeye salmon in the Karluk system declined 90% from 1894 to the 1950s and then increased 30% during the 1990s. Proposed causes for the decline include the following: initial overharvesting of the entire run which led to declines in the spawning population (Barnaby 1944); overharvesting of the middle part of the salmon run (Thompson[2]); intense predation on juvenile salmon by Dolly Varden (*Salvelinus malma*) and charr (*Salvelinus alpinus*) in freshwater (Rounsefell, 1958); loss of marine-derived nutrients in the lake (Rounsefell, 1958); increased population size of threespine stickleback (*Gasterosteus aculeatus*), a freshwater competitor (McIntyre[3]); asynchrony of the plankton bloom and fry emergence (Koenings and Burkett, 1987); and ocean climate change (Finney et al., 2000). The analysis of salmon-derived nitrogen levels in lake sediment cores revealed that from 1752 to 1993, nitrogen levels oscillated in synchrony with Gulf of Alaska sea surface temperatures reconstructed from tree ring widths (Finney et al., 2000). In our study, we found that Karluk sockeye salmon abundance had undergone significant fluctuations in association with C-O conditions and regimes.

Study area

The Karluk Lake and River system is located on the west side of Kodiak Island in the northern Gulf of Alaska (Fig. 1). Karluk River is about 36 km long and from 18 to 165 m wide. Karluk Lake is 19 km long and has an average depth of 48 m. Sockeye salmon spawn in the river and the lake, and their tributaries. The early-run (June 1–July 21) sockeye salmon spawn mostly in tributaries of the lake. Scales were collected from adult sockeye salmon returning from the ocean and were sampled at the weir located on the river. The weir was located near the mouth of the river from 1921 to 1941, 20 km upriver from the mouth from 1942 to 1944, and 300 m below the lake from 1942 to 2000. The average annual escapement was 421,146 sockeye salmon, 233,779 Chinook salmon (*O. tshawytscha*), 630,176 pink salmon, 12,867 coho salmon, 51 chum salmon, and 1,800 steelhead trout (*O. mykiss*), for the years 2006–2008 (ADF&G, 2009).

Northeastern Pacific salmon distribute and migrate primarily in the GOA and central North Pacific Ocean (Myers et al., 1996). The GOA continental shelf waters encompass an area of 37,000,000 km^2 (Burrell, 1986). The GOA is dominated by counterclockwise current systems in offshore waters (i.e., Alaska Current) and on the continental shelf (i.e., Alaska Coastal Current). Water moves parallel to shore at speeds of 13 to 133 cm/s (Reed and Schumacher, 1986).

Hypotheses and possible mechanisms that affect growth and survival

To explain mechanisms for climate regime conditions to affect growth and survival we hypothesized that 1) faster growth and higher-than-average brood-year survival was expected as a consequence of the warm C-O regimes from 1922 to 1946 and from 1977 to 2000, and slower growth and lower-than-average brood survival was expected as a consequence of the cool C-O regime from 1947 to 1976; 2) annual growth and variation in brood survival were expected to correlate positively with sea surface temperature, precipitation, and atmospheric circulation (Atmospheric Forcing Index), and to correlate negatively with upwelling and the bifurcation of the Pacific Current (Northern Oscillation Index); and 3) annual growth was expected to correlate positively with annual variations in brood survival for Karluk sockeye salmon.

Mechanisms were proposed for the influence of C-O indices on increased marine growth and brood survival of salmon. Sea surface warming during the spring initiates thermal stratification of the water column and an algal bloom in the Gulf of Alaska, food for the zooplankton that salmon feed on. Therefore, warmer sea surface temperatures in the spring were expected to increase the growth and survival of salmon. Annual primary productivity in the GOA is also limited by inorganic nitrogen, phosphorus, and silicon (Martin and Gordon, 1988). Winter and spring precipitation runoff from land to sea brings terrestrial silicon and iron into the GOA (Burrell, 1986). Precipitation also accelerates the Alaska Coastal Current (ACC) and draws nutrients in the GOA from the southern waters (Royer, 1979). Winter and spring downwelling aids in deep-mixing of inorganic nutrients into the euphotic zone to enhance the bloom of phytoplankton during the spring. The Atmospheric Forcing Index (AFI) and the Northern Oscillation Index (NOI) represent two additional pathways for nutrient input into the GOA. A positive

[2] Thompson, W. 1950. Some salmon research problems in Alaska. Presented at Alaskan Science conference of the National Academy of Science, National Research Council, Washington, 9–11 Novemeber, 1950. University of Washington, Fisheries Research Institute, Seattle, WA, 39 p.

[3] McIntyre, J. 1980. Further consideration of causes for decline of Karluk sockeye salmon. Unpubl. report, 29 p. .S. Fish and Wildlife Service, National Fisheries Research Center, Seattle, WA.

AFI is a measure of increased offshore upwelling of nutrient-rich, cool water in the central North Pacific, more frequent and intense storms passing through the GOA, increased frequency of westerly and southwesterly winds, and warmer coastal waters in the eastern North Pacific Ocean (McFarlane et al., 2000). The NOI describes the pattern of wind and atmospheric circulation between the North Pacific High (35°N, 130°W) and Darwin, Australia (10°S, 130°E) (Schwing et al., 2002). A more negative NOI is associated with a stronger northward projection of the Pacific Current along the Canada and southeast Alaska coasts that increases the speed of the ACC and draws nutrients and warmer waters from southern to northern coastal waters of Alaska.

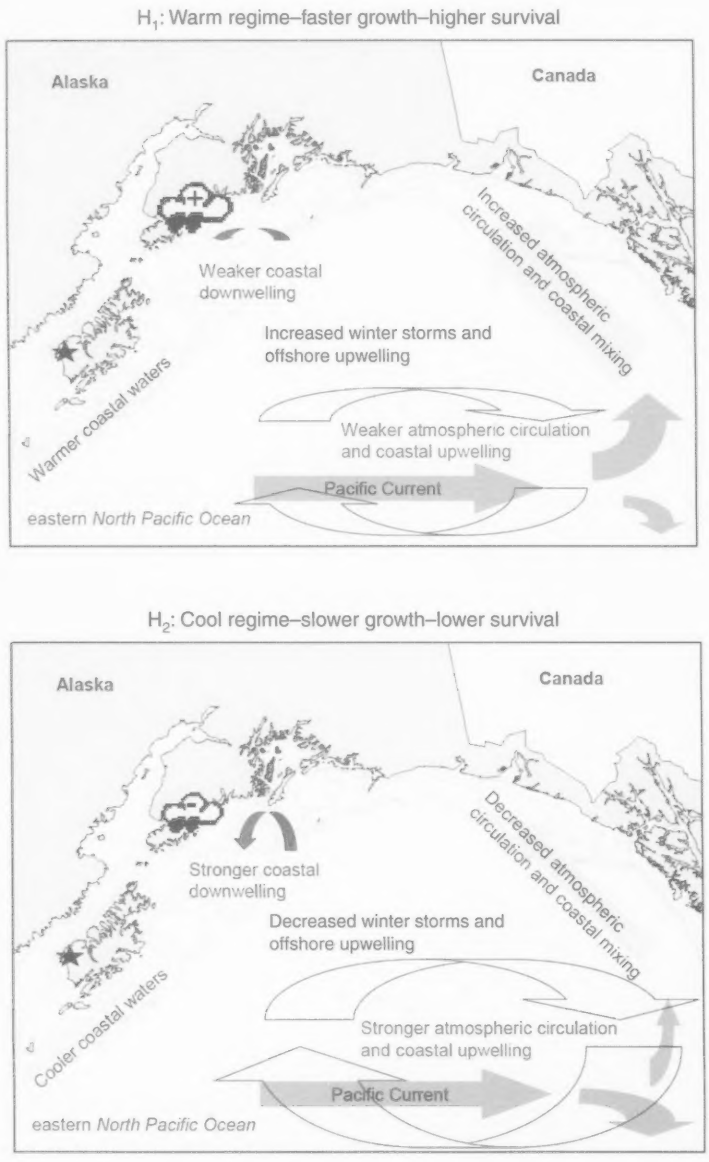

Figure 1

Schematics of the ocean-regime hypotheses (H_1 and H_2) for the effect of climate on the growth and survival of sockeye salmon from the Karluk system during warm and cool climatic and oceanic (C-O) regimes in the eastern North Pacific Ocean. A star marks the location of the Karluk River and Karluk Lake on Kodiak Island. The + sign represents higher precipitation amounts and – sign represent lower precipitation amounts.

Material and methods

Data, sample sizes, and assumptions

Measurements from scales from age-2.2 sockeye salmon were used to indirectly estimate the freshwater and marine growth rates of smolts and postsmolts. The age-2.2 adult fish had returned to the Karluk system during the early run spawning migration from May 1 to July 21 from 1924 to 2000. Seven years of data were unavailable (1945, 1947, 1958, 1965, 1966, and 1979). Scales were systematically selected from the archived collection of scales from the early-run of each year. The scales had been mounted on gum cards in the field. In the laboratory, impressions of the scales were made on plastic acetate cards (Arnold, 1951). Scale impressions were viewed with an Indus microfiche reader, model 4601-11 (Indus, West Salem, WI) with a 24× objective lens. Scale images were copied from the reader screen with a Screenscan Microfiche PC high-resolution scanner (Indus, West Salem, WI) and saved as tiff files by using ScreenScan Application software, vers. 1.00.0.8 (Indus, West Salem, WI). Images were then imported into the Optimate image analysis program (Media Cybernetics, Bathesda, MD) for measurement.

One scale was measured per fish, and 30–50 scales were measured from each year (n=70 years) for a total of 3167 scales read. Scales with evidence of resorption or regeneration were not measured. Measurements were taken along a consistent reference line drawn from the focus to the edge of the scale along the longest anterior radial axis to reduce the amount of variation in measurements at different radial axes (Martinson et al.,

2000). Marks were made along the reference line at the center of the scale focus, between the last freshwater circulus and the first marine circulus, at the outer edge of the first marine annulus, at the outer edge of the second marine annulus, and the outer edge of the scale. Scales were measured to the nearest 0.0001 mm.

Growth indices

Four growth rates in body length were estimated from the scale measurements. Body length at the time of entry into saltwater (FW) was estimated as the distance from the focus to the end of the last freshwater annulus or the end of the plus growth. First-year marine (M1) scale growth was estimated as the distance from the center of the space between the last freshwater and first marine circulus to the outer edge of the first marine annulus. Second marine-year scale growth (M2) was estimated as the distance from the outer edge of the first marine annulus and the outer edge of the second marine annulus. Third marine-year (M3) scale growth was estimated as the distance from the outer edge of the second marine annulus to the outer edge of the scale. Means were calculated for each growth variable by brood year. Growth along the radius of the scale was assumed to be proportional to the growth in length of the fish, where the distance from each annulus on the scale provides an estimate of the total annual increase in body length for each year at sea (Dahl, 1909).

Climate and oceanic indices

Five climatic and oceanic indices (C-O indices) were used in the analysis. Three coastal indices of the northern GOA included sea-surface temperature (SST), precipitation (PREC), and the Upwelling Index (UI). Two North Pacific Ocean wide indices were used: the Atmospheric Forcing Index (AFI) and the Northern Oscillation Index (NOI). Because of the shorter time series of the coastal indices, we used two periods for the correlation analysis (1922–2000 and 1951–2000).

Sea-surface temperature Sea-surface temperature (SST) was represented by the Reynolds reconstructed sea surface temperatures in waters off the continental shelf at a point 150 nmi south of the west end of Kodiak Island (55°N, 155°W), the general direction of migration of juvenile salmon. Data were accessed from the NOAA Pacific Fisheries Environmental Laboratory (PFEL), National Marine Fisheries Service, at the Southwest Fisheries Science Center, Pacific Grove, California (http://www.pfeg.noaa.gov, accessed June 2004). An average annual spring SST index was estimated as the average monthly (Mar–May) temperature (°C) for years from 1951 to 2000 (n=50 years).

Precipitation Precipitation (PREC) amounts (cm) near Kodiak, Alaska, were accessed from the NOAA Western Regional Climate Center homepage (http://www.wrcc.dri.edu, accessed June 2004). Daily records taken at the Kodiak Naval Air Station were available for the years 1951 to 1969 (n=19 years), data taken at the National Weather Service Office on the U.S. Coast Guard Kodiak Base were available for the years 1970 to 1972 (n=3 years), and data from the the Kodiak Airport were available for the years 1973 to 2000 (n=28 years). The PREC index was calculated as the cumulative winter and spring precipitation amounts from December 1 through May 31.

Upwelling Index The coastal upwelling index (UI) east of Kodiak Island (60°N, 149°W) from 1951 to 2000 (n=50 years) were obtained from the PFEL homepage (http://www.pfeg.noaa.gov, accessed June 2004). Upwelling indices to the east were correlated with the one used in our study. The UI values ($m^3/s/100$ m coastline) were derived from wind stress and resulting mass transport of the surface water from subsurface layers. Mass transport was determined by the wind stress divided by the Coriolis parameter (a function of the rotation and latitude of the earth). Positive UI values indicated upwelling and negative UI values indicated downwelling. Cumulative winter and spring coastal upwelling east of Kodiak Island were calculated as the sums of monthly values from December 1 to March 31.

Atmospheric Forcing Index The Atmospheric Forcing Index (AFI) was accessed from the Fisheries and Oceans of Canada fisheries climatology webpage (http://www.pac.dfo-mpo.gc.ca/sci/sa-mfpd/climate/clm_indx_afi.htm, accessed June 2004). The AFI values are the standardized scores of the first principle component of the winter (December through March) Aleutian Low Pressure Index, the Pacific Decadal Oscillation Index, and the Pacific Circulation Index (McFarlane et al., 2000). We used AFI winter values from 1922 to 2000 (n=79 years).

Northern Oscillation Index The Northern Oscillation Index (NOI) values were obtained from the PFEL homepage (http://www.pfeg.noaa.gov, accessed June 2004). The NOI is calculated as the difference between the monthly sea level pressure anomaly at the North Pacific High and the monthly sea level pressure anomaly at Darwin, Australia. The NOI was calculated as the average of the December through February from 1949 through 2000 (n=52 years).

Survival index

Direct estimates of marine survival of Karluk sockeye were not available; therefore indirect survival estimates were obtained from residuals of the stock-recruitment curve (Ricker, 1975). Stock size was estimated as the escapement counts of sockeye made at the Karluk weir from 1924 to 1996 (n=72 years), and recruitment was estimated as the number of offspring from the spawning population that returned from the ocean to the

Karluk commercial fishery and Karluk weir. Harvest, escapement, and age composition were available from the National Archives in Anchorage, the Alaska Department of Fish and Game, the National Marine Fisheries Service, and the Fishery Research Institute at the University of Washington.

With this approach, residuals (ε_i) of the fitted Ricker curve represented survival in the form of the annual deviation from the expected numbers of recruits based on the numbers of parents (escapement). The linear form of the survival model is given as

$$\ln\left[R_{t+r}/E_t\right]=\alpha+\beta E_t+\varepsilon, \quad (1)$$

where E = number of sockeye salmon counted at the Karluk weir during the spawning migration in year t;
R = number of sockeye salmon counted at Karluk weir and caught in the fishery in years r from brood year t;
α = density-independent parameter;
β = density-dependent parameter; and
ε = residuals, the survival index.

The survival index was lagged to match each of the three years of data of marine residence (i.e., juvenile, immature, and maturing stages) for the age 2.2 fish in the brood and was related to annual scale growth measurements and C-O indices.

The residual method is unfortunately less reliable than direct measurements of marine survival and requires several assumptions. One assumption is that the survival variability of the brood represents the survival of age 2.2 fish, a dominant age class in the brood. Also, because the recruitment estimate is based on the number of salmon counted at the Karluk weir and the number of salmon captured in the commercial fishery near the mouth of the Karluk River during the early and late run, it was assumed that there was equal fishing effort, minimal catch of non-Karluk sockeye salmon, and limited size selectivity of the fishing gear between runs and among years. It was also assumed that the fitted curve accurately reflected the overall density-dependent response of a stock at a given environmental state.

Analytical techniques

Ocean regime trends and comparison of regimes To describe and visually assess the low-frequency fluctuation in growth, climate indices, and survival in relation to the three ocean regimes, we fitted a loess regression line to the annual values using SigmaPlot software (Systat Software, Inc., Chicago, IL). The loess method of smoothing is based on tricube weighting and polynomial regression (Cleveland and Devlin, 1988). The loess regression method uses a variant of the local regression algorithm to approximate a nonlinear surface. Assume a series x_i for i=1, n. . The basic idea involves estimating a smoothed series y_i

$$y_i = g(x_i)+\varepsilon_i, \quad (2)$$

where points in the near neighbor of (y_i, x_i) have more weight than points further away.

In contrast use of an ordinary least-squares regression method, all points are equally weighted. In the analysis it is assumed that 30% of data points are used to compute each smoothed value and a 1° polynomial is then estimated. To determine the differences among regimes, an analysis of variance (ANOVA) was used to compare an average of the annual means of growth among C-O regimes. When a difference occurred we used the Tukey and Dunn pairwise comparison test to determine the regimes that differed.

Correlation analyses To describe the relationships among the scale growth, the C-O indices, and survival, we used the Pearson product moment correlation method. The null hypotheses of no associations among indices (H_0: ρ=0) was tested against the alternative hypotheses that growth, climate, and survival were positively (H_a: ρ>0) or negatively (H_a: ρ<0) related at a 5% level of significance (α=0.05). A Bonferonni correction factor was used to adjust the critical P-value for the number of correlations = 0.05/ the number of correlations. Correlations among growth variables within broods were used to determine the dependence of growth on growth in the previous year.

Results

Influence of C-O regimes on mean sockeye salmon growth

Juvenile (M1) growth and immature (M2) growth were lower during the cool regime and higher during the recent warm regime, whereas maturing growth (M3) was lower during the early warm regime and higher during the cool and recent regime (Fig. 2). Growth varied over time by 14% for FW, 10% for M1, 15% for M2, and 46% for M3. FW declined 12% during the early warm regime, was relatively constant during the cool regime, increased 8% from 1970 to 1980, and decreased 8% from 1980 to 1998. M1 decreased during the early warm regime, increased 7% from the later part of the early warm regime to mid-cool regime, increased 5% from 1970 to 1980, and decreased 2% from 1980 to 1998. M2 was low and constant during the early warm and cool regimes and increased 15% from the early 1970s to the mid 1980s. M3 increased 40% from the mid 1920s to the mid-cool regime period and decreased 18% in the mid-1980s.

For all four growth variables, at least one mean or median value was significantly different (ANOVA: P<0.002) among periods (Table 1). Mean M1 and M2 were significantly higher (Tukey test: P<0.05) during the 1977–2000 warm C-O regime than during the 1922–46 warm and 1947–76 cool regimes. Mean FW

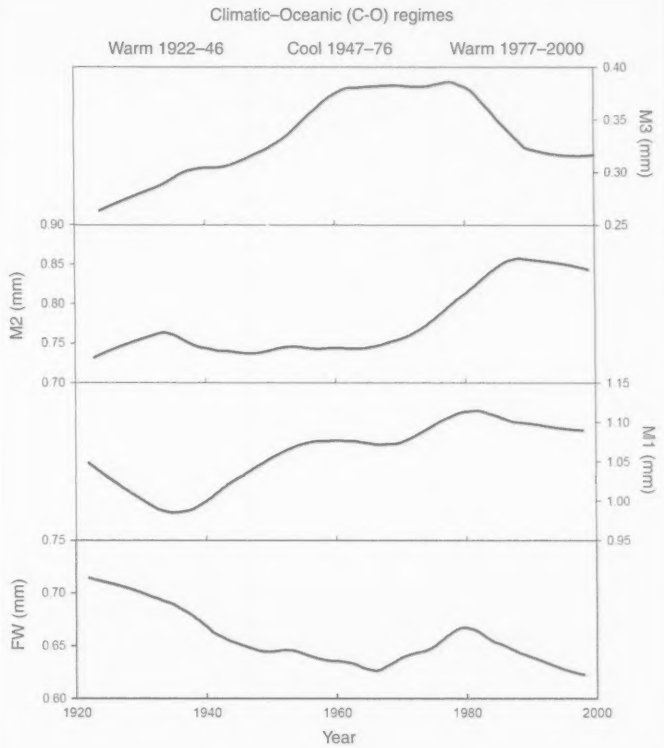

Figure 2

Trends in annual freshwater (FW) and annual (first year through third year, M1–M3) marine growth for the 1922–46 warm, 1947–76 cool, and 1977–2000 warm regimes represented by loess regression lines. Scale measurements are estimates of growth rates for age 2.2 sockeye salmon (*Oncorhynchus nerka*) that returned to Karluk River during the early run spawning migration from 1924 to 2000.

Table 1

Comparison of freshwater and oceanic growth rates of sockeye salmon (*Oncorhynchus nerka*) that returned to the Karluk lake and river system, Alaska, from years 1924 to 2000. Smolt length (freshwater, FW), and juvenile (first-year marine, M1), immature (second-year marine, M2), and maturing (third-year marine, M3) growth were estimated from measurement on the scales of age-2.2 sockeye salmon that returned to the Karluk system, Kodiak Island, Alaska. "Yes" indicates normal distribution and equal variances of the growth variables. Significance differences occurred at $P \leq 0.05$ for normality, variance, ANOVA, and multiple comparison tests.

	Assumption test		ANOVA		Tukey and Dunn pairwise test mean or median and statistical differences				
Growth variable	Normal	Equal variance	Test statistic	*P*-value	1922–1946 Warm regime		1947–1976 Cool regime		1977–2000 Warm regime
FW	Yes	Yes	6.624	0.002	0.687	>	0.640	=	0.644
M1	Yes	Yes	28.753	<0.001	1.006	<	1.072	<	1.104
M2	Yes	Yes	24.625	<0.001	0.745	=	0.752	<	0.841
M3	Yes	No	26.055	<0.001	0.293	<	0.366	=	0.353

was significantly higher ($P \leq 0.05$) during the 1922–46 warm regime than during the 1947–76 cool, and 1977–2000 warm regimes. Mean M3 was significantly higher (25%; Dunn's test, nonparametric test; $P \leq 0.05$) during the 1947–76 cool regime than during the 1922–46 warm regime but not significantly higher ($P > 0.05$) than the mean M3 during the 1977–2000 warm regime. Growth variables within a brood were not significantly correlated (Table 2).

The regional C-O indices in the North Pacific varied in association with the warm (1922–46), cool (1947–76), and warm (1977–2000) C-O regimes (Fig. 3). Compared to the 1947–76 cool regime, the 1977–2000 warm regime had 1°C higher SST, a 54% higher PREC, weaker coastal downwelling near Prince William Sound, 42% higher AFI, and 61% lower NOI. Compared to the 1947–76 cool regime, the 1922–46 warm regime had a 27% higher PREC and a 47% higher AFI.

Influence of regional C-O conditions on mean sockeye salmon growth

Regional C-O indices correlated significantly with juvenile (M1) and immature (M2) scale growth, but not with maturing growth (M3) (Table 3). Growth was correlated with C-O indices for the 1951–2000 period, but not with C-O indices for the 1922–2000 period. Growth indices were correlated with two of the five C-O indices. M1 had a significant positive association with AFI (r=0.40; P=0.005). M2 had a strong positive relationship with the PREC (r=0.39; P=0.005). FW and M3 did not correlate with the five C-O indices.

Interrelationships among regional C-O conditions, mean growth, and annual survival of sockeye salmon

Survival, as indicated by the residuals from the stock-recruitment model, was higher than expected during warm regimes and lower than expected during the cool regime (Fig. 4), and there was a brief high from 1959 to 1965. The linear regression model of the logarithm of the recruits per spawner [$\ln(R/E)$] as a function of spawners (E) was

$$\ln(R_{t+r} / E_t) = -0.48039 \times 10^{-7} \cdot E + 0.7668. \quad (3)$$

A reduction in the number of recruits per escapement at higher escapement levels is characteristic of strong density-dependence at high stock levels (r^2=0.1035; P=0007). Residuals of the model were used as an index for survival.

Survival was not significantly correlated with growth (Table 4), but it was significantly correlated with four of the five C-O indices and with C-O indices during all marine life stages (Table 5). For the 1921–89 broods, survival was correlated positively with SST (0.409, 0.567, 0.536) and AFI (0.314, 0.307, 0.364) during the M1, M2, and M3 years spent at sea by the age-2.2 fish in the brood (Table 5). For the 1948–89 brood years, survival correlated positively with SST (0.409, 0.607), PREC (0.397, 0.349), and AFI (0.447, 0.477) during the first and second year at sea. A significant negative correlation of 0.421 occurred between UI and survival in the final year at sea.

Table 2

Correlation coefficients (r) and P-values among growth variables for broods of age-2.2 sockeye (*Oncorhynchus nerka*) that returned as adults to the Karluk River weir during the early run. Scales were used for measurements. Smolt length (freshwater, FW), and juvenile (first-year marine, M1), immature (second-year marine, M2), and maturing (third-year marine, M3) growth were estimated from measurements of the scales of age-2.2 sockeye salmon that returned to the Karluk system, Kodiak Island, Alaska. There were no significant relationships between any pair of variables in the correlation table at 5% (P<0.008=0.05/6) and 1% (P<0.002=0.01/6).

Brood year	Growth variables		M1	M2	M3
1919–95					
n=71	FW	r	−0.150	−0.079	−0.214
		P	0.212	0.512	0.073
	M1	r		0.24	0.253
		P		0.060	0.033
	M2	r			0.009
		P			0.940
1948–95					
n=48	FW	r	0.287	0.055	0.065
		P	0.051	0.722	0.675
	M1	r		0.138	−0.098
		P		0.372	0.527
	M2	r			−0.291
		P			0.050

Discussion

Influence of C-O regimes on mean sockeye salmon growth

The synchronous patterns between sockeye salmon mean M1 and M2 growth indices and the two most recent ocean regimes may indicate that changes in C-O conditions in the North Pacific Ocean influenced the marine growth of sockeye salmon from Karluk River and Karluk Lake. The increase in juvenile and immature growth and the reduction of maturing growth in the final year at sea of sockeye salmon from the northern Gulf of Alaska after the 1976–77 regime shift is distinct from the growth pattern seen in sockeye salmon stocks from the Bering Sea off western Alaska. For western Alaska, the M1 of Bristol Bay sockeye salmon and M1 and M2 of Chignik sockeye salmon tended to increase soon after the 1976–1977 climate shift (Ruggerone et al. 2007). However, for

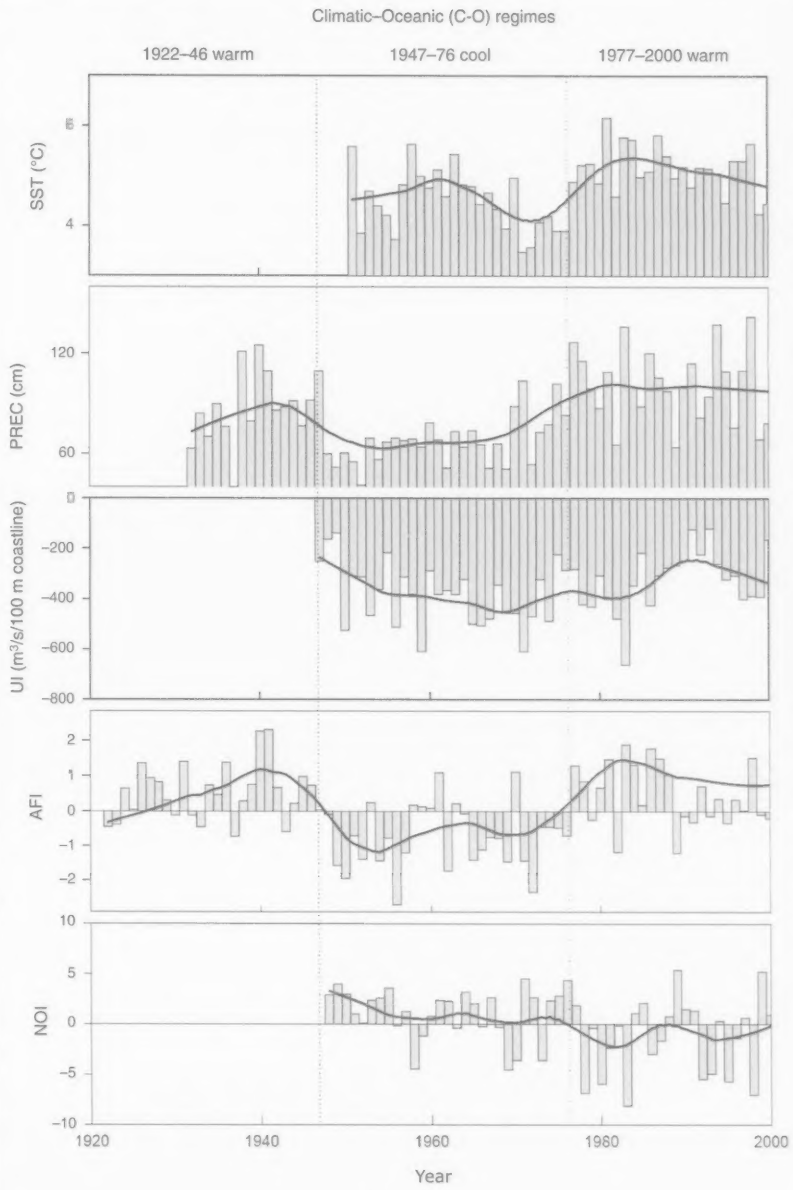

Figure 3

Trends in climate and ocean conditions as represented by five climatic and oceanic (C-O) indices in the eastern North Pacific Ocean from 1922 to 2000. C-O indices comprise the average spring (Mar–May) coastal sea-surface temperature (SST), cumulative amounts of winter and spring (Dec–May) coastal precipitation (PREC), cumulative winter (Dec–Mar) coastal Upwelling Index (UI), average winter (Dec–Mar) Atmospheric Forcing Index (AFI), and the average winter (Dec–Feb) Northern Oscillation Index (NOI). Lines are the loess regression line. Bars are the annual means.

age 2.2 sockeye salmon from Kvichak River in western Alaska from 1920 to 1997, a positive warm-regime effect occurred for M1, but not for M2 (Isakov et al., 2000). For ocean age-3 sockeye salmon from Bristol Bay from 1955 through 2000, scale growth increased after 1977 (following entry into saltwater and later in the year for M1, after the peak summer growing season for M2, and during the peak growing season for M3 (Ruggerone et al., 2005). Such long-term patterns may continue to persist over a period of years during which C-O regime conditions remain relatively stable or warm.

Several mechanisms could explain the lower than expected M1 and M2 growth during the 1922–46 warm regime. First, growth rates derived from the scales of returning adult salmon, a small subset of the original smolts entering the ocean, may not accurately reflect the actual growth rate of the population of juvenile and immature salmon as a whole. However, the decline in freshwater scale growth in the mid 1940s corresponds with the reduced freshwater productivity in Karluk Lake over the period 1940s through 1993, as indicated by salmon-derived nitrogen levels in lake sediment cores from 1752 to 1993 (Gregory-Eaves et al., 2004), and the decline in scale growth supports the use of adult scales to estimate the past growth life history of the fish. Second, higher marine survival of smaller size classes indicate a larger proportion of slower growing fish in the surviving population that was sampled. Alternatively, an increase in inter- and intra-specific competition for food led to slower M1 and M2 growth during the 1922–46 warm regime. Finally, a change in the scale sampling method in the 1950s indicated a lower precision and accuracy of growth measurement in the years before the 1950s. Before the 1950s, a scraping method was used to remove scales

Table 3

Correlation coefficients (r), P-values, and sample sizes (n) relating the growth of early run age-2.2 sockeye (*Oncorhynchus nerka*) from the Karluk system to climatic and oceanic (C-O) indices in the North Pacific Ocean. C-O indices include the average spring (Mar–May) coastal sea-surface temperature (SST), cumulative winter and spring (Dec–May) coastal precipitation amounts (PREC), cumulative winter (Dec–Mar) coastal upwelling (UI), average winter (Dec–Mar) Atmospheric Forcing Index (AFI), and the average winter (Dec–Feb) Northern Oscillation Index (NOI). Smolt length (freshwater, FW), and juvenile (first-year marine, M1), immature (second-year marine M2), and maturing (third-year marine, M3) growth were estimated from measurement on the scales of age-2.2 sockeye salmon. Relationships between variables in the correlation table are significant at 5% (P<0.01=0.05/5) indicated by *.

Year	Growth variable	C-O indices for the North Pacific Ocean				
		SST	PREC	UI	AFI	NOI
1922–2000						
FW	r	0.245	0.070	−0.101	0.224	0.025
	P	0.109	0.590	0.493	0.061	0.870
	n	44	61	48	71	47
M1	r	0.303	0.158	0.115	−0.074	−0.089
	P	0.047	0.223	0.438	0.538	0.552
	n	44	61	48	71	47
M2	r	0.194	0.283	0.269	0.151	−0.269
	P	0.201	0.026	0.061	0.209	0.064
	n	45	62	49	71	48
M3	r	−0.193	−0.151	−0.176	−0.222	0.017
	P	0.200	0.237	0.225	0.056	0.908
	n	46	63	49	71	49
1951–2000						
FW	r	0.245	0.120	−0.118	0.206	−0.001
	P	0.109	0.439	0.444	0.181	0.994
	n	44	44	44	44	44
M1	r	0.303	0.269	0.210	0.380*	−0.060
	P	0.047	0.079	0.171	0.011	0.700
	n	44	44	44	44	44
M2	r	0.194	0.392*	0.250	0.308	−0.251
	P	0.201	0.008	0.097	0.039	0.096
	n	45	45	45	45	45
M3	r	−0.193	−0.248	−0.214	−0.104	0.077
	P	0.200	0.097	0.153	0.490	0.612
	n	46	46	46	46	46

from a fish and involved a wider area of the body of a fish than the more localized sampling with forceps done after the 1950s.

The inverse relationship between the regime pattern and M3 is consistent with the idea that a density-dependent effect on growth occurred during the final year at sea. For example, mean body weights of pink, chum, and sockeye salmon stocks in commercial fisheries ranging from Washington to western Alaska were negatively related to increased salmon production from the mid-1970s to the mid-1990s (Bigler et al., 1996; Helle et al., 2008). Declines in size of chum salmon were also documented for carcasses sampled at Fish Creek, Hyder, Alaska, and Quilcene National Fish Hatchery in Hood Canal, Washington (Helle and Hoffman, 1995). Authors speculated that a greater number of fish in the ocean led to increased competition for food and in turn decreased the marine growth and size at maturity of salmon.

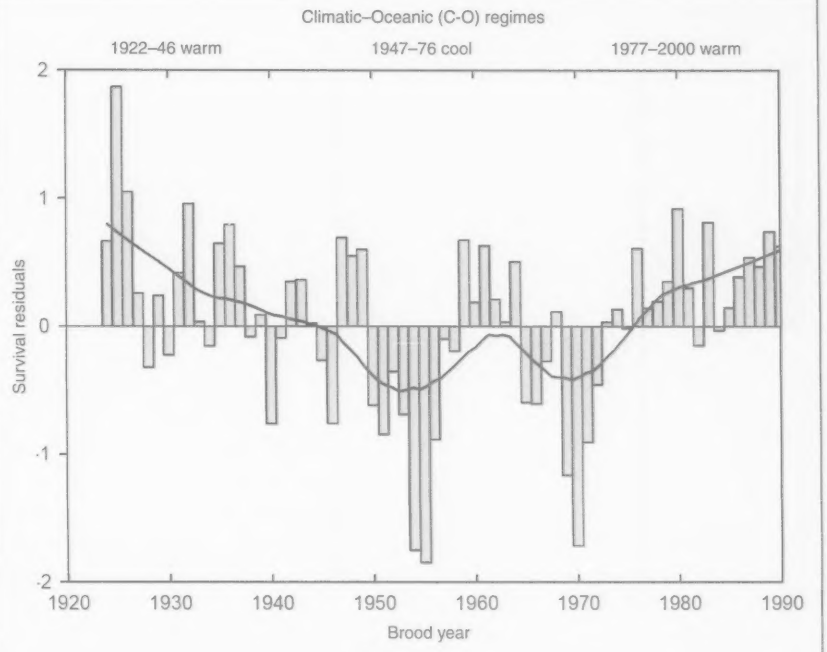

Figure 4

Annual survival as indicated by residuals from a stock-recruitment curve ($\ln[R_{t+2}/E_t]=\alpha+\beta E_t$) for sockeye salmon (*Oncorhynchus nerka*), from Karluk Lake, Alaska, 1924 to 1989. The line is the loess regression line. Bars are the annual values.

Influence of regional C-O conditions on mean sockeye salmon growth

Juvenile growth was correlated with atmospheric circulation during the winter and spring and weakly correlated with sea surface temperature and amount of coastal precipitation, whereas immature growth was correlated with coastal precipitation and weakly correlated with sea surface temperature and atmospheric circulation. Results are similar to those from other studies. For sockeye salmon that returned to the Kvichak River in western Alaska, M1 was positively correlated with SST in Bristol Bay and near the Aleutian Islands in the eastern Bering Sea (Isakov et al., 2000). Similarly, Helle (1979) found a significant relationship (r=0.64; P<0.01) between the mean number of circuli formed during the first year of marine growth on the scales of age-0.3 chum salmon from Olsen Bay in Prince William Sound, Alaska, and the mean summer and fall SST, but not air temperature, cloud cover, PREC, atmospheric pressure, or seawater density, in the GOA from 1957 to 1975. For the same Karluk sockeye salmon time series, the early juvenile growth, as indicated by the distance from between the last freshwater and first marine circulus to the end of the 9th circulus, was negatively correlated with the numbers of juvenile pink salmon fry (r=–0.77; P=0.002) released from hatcheries in Prince William Sound, Cook Inlet, and Kodiak, and the distance from the 9th circulus to the end of the first marine annulus within M1 was positively correlated with summer SST in the GOA (r=0.49; P<0.01) (Martinson, 2004). A stronger density-dependent effect on early marine growth indicates a masked influence of C-O conditions on growth (Martinson et al., 2008).

Interrelationships among regional C-O conditions, mean growth, and annual survival of sockeye salmon

The higher survival during the warm regimes and lower survival during the cool regime indicate that marine climate influenced the production of sockeye salmon from Karluk Lake. Results are consistent with other studies showing a strong relationship between various C-O indices and salmon survival. Regional scale covariation in survival rates of pink, chum, and sockeye salmon were more closely related to coastal SST during the first summer at sea than surface salinity or upwelling

(Mueter et al., 2002b). In addition, coastal SST during the first year at sea for sockeye salmon and within 400 km of the point of ocean entry associated positively with survival rates of 19 stocks ranging from western Alaska to northern British Columbia and negatively with survival rates of 18 stocks in central British Columbia and Washington (Mueter et al., 2002a). Warmer winter SST (Nov–Feb) in offshore waters in the Gulf of Alaska were related to increases in total annual adult catch plus escapement of major B.C. and Alaska stocks (Pyper and Peterman, 1999). For the Karluk sockeye salmon, survival was correlated with spring coastal sea surface temperatures, winter and spring precipitation, and the more stormy winters before and following the juvenile stage, indicating two important marine periods in determining the survival of Karluk sockeye salmon. This result is consistent with the hypotheses that climatic and oceanic conditions during the first few months at sea (Parker, 1971) and the first winter at sea (Beamish and Mahnken, 2001) are important in determining mortality rates.

Climatic processes influencing salmon in the Pacific Northwest were not consistent with processes affecting Karluk sockeye salmon from Alaska. For example, water column mixing, as indicated by the Upwelling Index, did not correlate positively with faster growth or higher survival of Karluk sockeye salmon except in the winter and spring before the year of maturity. In the coastal waters off Oregon, by contrast, upwelling during the first year at sea of age-1 coho salmon was positively correlated with commercial catch of coho salmon from 1947 to 1962 (Scarnecchia, 1981). The Northern Oscillation Index that is associated with a stronger trade winds, stronger coastal upwelling off Washington and Oregon, cooler surface and subsurface sea temperatures in waters off Washington, and higher Oregon hatchery salmon production (Schwing et al., 2002) was not correlated with the growth and survival of Karluk sockeye salmon.

The lack of a correlation between survival and growth of the age-2.2 sockeye salmon is similar to the findings of other studies where growth was determined from the scales of adult sockeye salmon. For example, M1 on the scales of adult sockeye salmon that returned to Egegik and Kvichak rivers in Bristol Bay, Alaska, was not correlated with variations in brood survival estimated from residuals of the Ricker model (Farley et al., 2007). From 1977 to 1997, growth on the scales of adult sockeye salmon from Bristol Bay showed reduced M2 and M3 growth and there was a smaller size-at-age for adults and lower smolt-to-adult survival rates for the even-year migrating smolts (Ruggerone et al., 2003). Alternatively, the size of juvenile sockeye salmon in Bristol Bay was positively correlated with survival rates of Bristol Bay sockeye salmon (Farley et al., 2007). The difference in results of correlating juvenile size with survival and with correlating adult scale growth with survival is likely due to size-selective mortality on the smaller fish during the juvenile stage (Farley et al., 2007; Moss et al., 2005) and a reduced detection of growth-survival relationships based on adult scales.

Table 4

Correlation coefficients (r), P-values, and sample sizes (n) relating survival to growth of sockeye salmon from Karluk River and Karluk Lake, Kodiak Island, Alaska. Survival is the residuals of the Ricker spawner-recruitment curve for Karluk sockeye salmon (*Oncorhynchus nerka*). Smolt length (freshwater, FW), and juvenile (first-year marine, M1), immature (second-year marine, M2), and maturing (third-year marine, M3) growth were estimated from measurement on the scales of age-2.2 sockeye salmon. No relationships between any pair of variables are significant at 5% ($P<0.0125=0.05/4$) indicated by *.

Brood year		Growth variable			
		FW	M1	M2	M3
1921–89		0.073	0.005	0.136	−0.290
Survival	r	0.035	0.315	0.301	−0.106
	P	0.573	0.967	0.294	0.022
	n	62	62	62	62
1948–89					
Survival	r	0.035	0.315	0.301	−0.106
	P	0.835	0.058	0.070	0.531
	n	37	37	37	37

Conclusion

The first-, second-, and third-year marine scale growth, a proxy for the change in growth rate, of Karluk sockeye salmon was not detected as a link between climate and survival for Karluk sockeye salmon. If the growth on the scales of adult sockeye salmon represents only a subset of the survivors (possibly fewer smaller and slower growing fish when mortality is high), then the weaker climate-growth-survival relationships recognized from adult scales provides insight into possible growth-climate-survival relationships. The growth-climate relationships and the mechanism for these relationships should further be assessed by relating the size and growth of juvenile salmon with physical and biological conditions measured at sea.

Acknowledgments

We thank the numerous agencies and individuals that collected information from sockeye salmon at the Karluk Research Station on Karluk River from 1924 to 2000. Information sources included R. Gard and R. Bottorff, authors of a book on Karluk Lake, the National Archives in Anchorage, the National Marine Fisheries Service in Juneau, the Fisheries Research Institute at the University of Washington, and M. Witteveen, M. Foster, and P. Nelson at the Kodiak office for the Alaska Department of Fish and Game.

Table 5

Correlation coefficients (r), P-values, and sample sizes (n) relating survival to climatic and oceanic (C-O) conditions of the North Pacific Ocean experienced by the age-2.2 sockeye salmon (*Oncorhynchus nerka*) from Karluk River and Karluk Lake during their juvenile, immature, and maturing stages at sea. Survival is the residuals of the Ricker spawner-recruitment curve of Karluk sockeye. C-O indices comprise the average spring (Mar–May) coastal sea-surface temperature (SST), cumulative winter and spring (Dec–May) coastal precipitation amounts (PREC), cumulative winter (Dec–Mar) coastal upwelling (UI), average winter (Dec–Mar) Atmospheric Forcing Index (AFI), and the average winter (Dec–Feb) Northern Oscillation Index (NOI). Relationships between any pair of variables in the correlation table are significant at 5% (P<0.010=0.05/5) and 1% (P<0.002=0.01/5), indicated by * and **, respectively.

Marine life stage		C-O indices for the North Pacific Ocean				
		SST	PREC	UI	AFI	NOI
1921–89 brood years						
juvenile	r	0.409*	0.214	0.300	0.314*	−0.140
	P	0.007	0.098	0.043	0.009	0.360
	n	42	61	46	69	45
immature	r	0.567**	0.154	0.349	0.307*	−0.112
	P	0.00007	0.234	0.016	0.010	0.458
	n	43	62	47	69	46
maturing	r	0.536**	0.240	0.258	0.364**	−0.138
	P	0.0002	0.059	0.077	0.002	0.357
	n	44	63	48	69	47
1948–89 brood years						
juvenile	r	0.409*	0.397*	0.215	0.447*	−0.175
	P	0.007	0.009	0.172	0.003	0.267
	n	42	42	42	42	42
immature	r	0.607**	0.349*	0.386	0.477**	−0.180
	P	0.00002	0.024	0.012	0.001	0.253
	n	42	42	42	42	42
maturing	r	−0.195	−0.178	−0.421*	−0.107	0.117
	P	0.189	0.232	0.003	0.474	0.435
	n	42	42	42	42	42

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Population connectivity among Dry Tortugas, Florida, and Caribbean populations of mutton snapper (*Lutjanus analis*), inferred from multiple microsatellite loci

Kathryn Shulzitski (contact author)[1]
Michael A. McCartney[1]
Michael L. Burton[2]

Email address for contact author: kshulzitski@rsmas.miami.edu

Present address: Marine Biology and Fisheries Division
Rosenstiel School of Marine and Atmospheric Science
University of Miami
4600 Rickenbacker Causeway
Miami, Florida 33149-1098

[1] Department of Biological Sciences
Center for Marine Science
University of North Carolina Wilmington
5600 Marvin Moss Lane
Wilmington, North Carolina 28409

[2] National Marine Fisheries Service
Southeast Fisheries Science Center
101 Pivers Island Road
Beaufort, North Carolina 28516

Abstract—Determining patterns of population connectivity is critical to the evaluation of marine reserves as recruitment sources for harvested populations. Mutton snapper (*Lutjanus analis*) is a good test case because the last known major spawning aggregation in U.S. waters was granted no-take status in the Tortugas South Ecological Reserve (TSER) in 2001. To evaluate the TSER population as a recruitment source, we genotyped mutton snapper from the Dry Tortugas, southeast Florida, and from three locations across the Caribbean at eight microsatellite loci. Both *F*-statistics and individual-based Bayesian analyses indicated that genetic substructure was absent across the five populations. Genetic homogeneity of mutton snapper populations is consistent with its pelagic larval duration of 27 to 37 days and adult behavior of annual migrations to large spawning aggregations. Statistical power of future genetic assessments of mutton snapper population connectivity may benefit from more comprehensive geographic sampling, and perhaps from the development of less polymorphic DNA microsatellite loci. Research where alternative methods are used, such as the transgenerational marking of embryonic otoliths with barium stable isotopes, is also needed on this and other species with diverse life history characteristics to further evaluate the TSER as a recruitment source and to define corridors of population connectivity across the Caribbean and Florida.

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The elucidation of patterns of population connectivity and the determination of sources of recruiting larvae are central concerns among ecologists and are critical for the implementation of spatially explicit management strategies. The marine environment provides a particularly complex backdrop for studies of connectivity because absolute barriers to dispersal are rare and ocean currents can be temporally and spatially heterogeneous. In addition, the majority of marine organisms have a pelagic larval phase, and because of the small sizes and patchy distributions of individuals at this stage, it is nearly impossible to directly observe dispersal events (Leis, 1991). Genetic markers have often been used to infer dispersal scale and connectivity (Hellberg, 2007). One advantage of this approach is that it provides information about effective dispersal among populations (i.e., only migrants that go on to reproduce in their new population will contribute gene copies). A second advantage is that population genetic structure reflects an average, over many generations, of migration events that are likely to vary substantially over time, and therefore it provides an estimate of population connectivity that is relevant over the long term.

Studies with genetic markers have increased our understanding of population connectivity in the Caribbean region. Shulman and Bermingham (1995) found weak but significant population subdivision across the Caribbean basin for three out of eight fish species using restriction endonuclease analyses of mitochondrial DNA (mtDNA). Taylor and Hellberg (2003) examined mtDNA haplotypes of the sharknose goby (*Elacatinus evelynae*) and demonstrated extremely restricted dispersal among populations. Analyses of genotypes at multiple microsatellite loci in elkhorn coral (*Acropora palmata*) indicated two distinct genetic groups, corresponding to western and eastern Caribbean sampling locations (Baums et al., 2005). In a recent study, Purcell et al. (2006) found weak genetic structure and yet a significant pattern of isolation-by-distance using microsatellite markers for the French grunt (*Haemulon flavolineatum*). In contrast, the bluehead

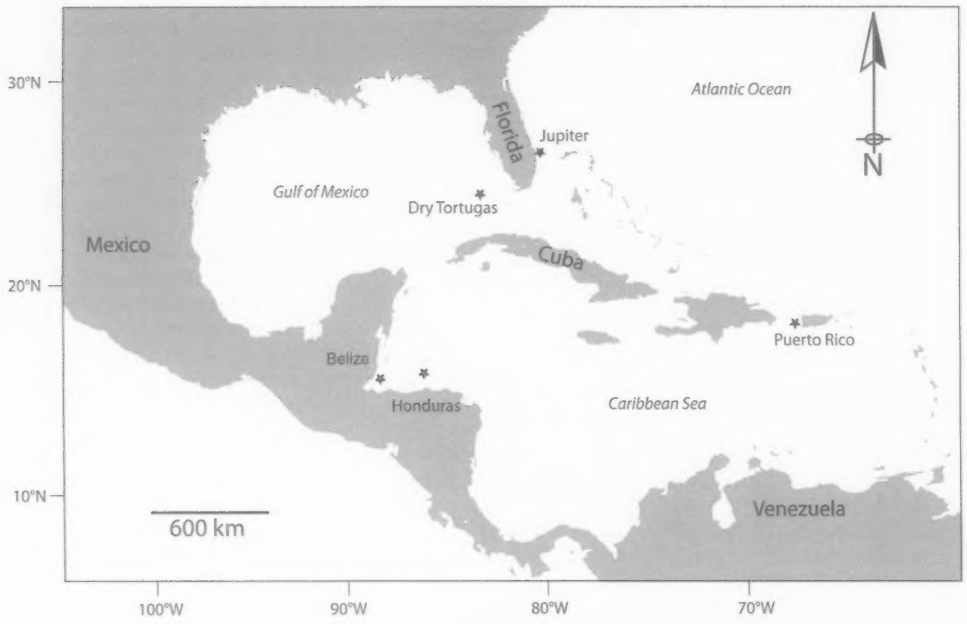

Figure 1

Map of sampling locations for mutton snapper (*Lutjanus analis*) collected across Florida and the Caribbean for microsatellite analysis. Stars denote sampling sites. Collection dates were as follows: Belize=May 2003; Dry Tortugas, FL=June–December 2003; Honduras=April 2004; Jupiter, FL=October 2004; and Puerto Rico=September 2004–February 2005. Adult fish were collected with hook and line; juvenile fish (Jupiter site only) were collected by seining.

wrasse (*Thalassoma bifasciatum*) lacks structure even at the scale of the entire Caribbean basin. Similarly, the slippery dick (*Halichoeres bivittatus*) shares mtDNA haplotypes across biogeographical provinces and locations separated by more than 2000 km (Rocha et al., 2005).

The results of these studies indicate similar opportunities for evaluating patterns of connectivity among reef fish populations in the Dry Tortugas, southeast Florida, and the Caribbean basin. In July 2001, the Tortugas South Ecological Reserve (TSER) was established approximately 110 km southwest of Key West, Florida, encompassing an historical fishing area known as Riley's Hump. This topographic feature was instrumental in the delineation of the reserve boundaries because it serves as a spawning site for various commercially and recreationally important snapper and grouper species (Lindeman et al., 2000). The high site fidelity and temporal stability exhibited by these spawning aggregations (Domeier and Colin, 1997) has led to their heavy exploitation and rapid decline on Riley's Hump (Burton, 2002). In fact, this site represents the last known major spawning aggregation for the mutton snapper (*Lutjanus analis*) in U.S. waters, making this species of particular interest to both conservationists and fishery managers.

In this study, we used *L. analis* as a focal species to examine eight high-resolution genetic markers to estimate connectivity among populations in the TSER, in southeast Florida, and at other sites throughout the Caribbean, with the ultimate goal of identifying larval source populations.

Materials and methods

Sample collection and genotyping

Mutton snapper tissue samples were obtained between May 2003 and February 2005 from five geographic locations and stored in salt-saturated dimethyl sulfoxide (DMSO). Samples of adult fish came from Gladden Spit, Belize (BZ); Roatan, Honduras (HN); Dry Tortugas (DT), Florida; and Mayaguez, Puerto Rico (PR) (Fig. 1); in addition, a sample of juvenile fish (standard length [SL]: 34–233 mm) from Jupiter (JP), Florida, was collected from Jupiter Inlet in October 2004 to serve as a downstream population. Genomic DNA was isolated according to a modification of the "rapid isolation of mammalian DNA" protocol of Sambrook and Russell (2000).

Two-hundred and forty-five individuals were genotyped at eight microsatellite loci (Table 1). Amplifications (15 µL) contained 5–20 ng template DNA, 15 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM each dNTP, 0.5 uM each primer, and 0.75 U

of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Cycling parameters began with a hot start of 10 minutes at 95°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at the optimized annealing temperature (see Table 1), and 60 seconds at 72°C, and a final extension of 30 minutes at 72°C. Polymerase chain reaction products were visualized on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA) and examined further with GENESCAN 3.7 software (Applied Biosystems, Foster City, CA). In GENOTYPER 3.7 (Applied Biosystems, Foster City, CA) peaks were labeled and binned into allele-size categories.

Data analysis

Each population was tested for departures from Hardy-Weinberg equilibrium (HWE) at each locus with GENEPOP vers. 3.4 software (Raymond and Rousset, 1995a) by using a probability test and a Markov chain method to obtain the unbiased exact *P*-value (Guo and Thompson, 1992). In a similar manner, all loci and population pairs were tested for linkage equilibrium. Because sample sizes varied from 40 to 55 individuals, allelic richness (the number of alleles present in populations independent of sample size) was calculated for each population-locus combination and overall with FSTAT vers. 2.9.3 software (Goudet, 1995). This parameter is an estimate of the expected number of alleles for a subsample of genes equal in size to that of the smallest sample.

In order to assess allelic and genotypic distributions across populations, an exact probability test (Raymond and Rousset, 1995b) and a log-likelihood based exact test (Goudet et al., 1996) were performed in GENEPOP to evaluate genic and genotypic differentiation, respectively. Both tests employed a Markov chain method to calculate an unbiased estimate of the *P*-value.

An unbiased estimator, θ (Weir and Cockerham, 1984), of Wright's (1921) fixation index (F_{ST}), a measure of among-population subdivision, was calculated with the GENETIX vers. 4.02 program (Belkhir et al., 2001) for each locus, as well as for each pairwise population comparison; permutation tests (1000 randomizations) were used to estimate *P*-values. An estimator of R_{ST}, ρ (Slatkin, 1995), was also calculated by using FSTAT (Goudet, 1995). This analog of F_{ST} takes into account allelic size by assuming that alleles of a similar size are more closely related, given that loci adhere to a stepwise mutation model (Slatkin, 1995). An additional measure of pairwise genetic differention, ϕ_{ST}, has been reported to be more appropriate in the case of highly polymorphic loci such as microsatellites, and was calculated with Geno-

Table 1

Mutton snapper (*Lutjanus analis*) microsatellite loci isolated for this study. T_m = annealing temperature; bp = base pairs; n = number of individuals genotyped; A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for Hardy-Weinberg equilibrium: * $P<0.05$, *** $P<0.001$.

Locus	Repeat motif of clone	Primer sequences (5' to 3')	T_m (°C)	Product size range (bp)	n	A	H_E	H_O
La25	$(ATCT)_{19}$	F: GGA GGA ACC TCC TGG AAT GT R: GTT TGC ACT TGA AGA AAA AGG GTG A	63	137–235	241	36	0.942	0.926
La39	$(TG)_{11}$	F: TGC TGA GGA GCA TTT GCT TT R: GTT TAA AGT CAC ATA AAC GGG GAC T	65	143–179	226	15	0.802	0.603***
La18a	$(ATGG)_6$ GTGGATGG$(ATAG)_{21}$	F: CCT CAC TTT TGT GTG AGA CAG C R: GTT ATG ATT GCT AGG AGC ATC TGG	65	188–340	239	37	0.964	0.963
La27a	$(AGAT)_{22}$	F: CTT AGC AAG CCA ACA AAC AAT G R: GTT TCC AAG GTC CAT TGA TCT TTA GTG	65	176–312	184	39	0.939	0.856*
La34a	$(AGAT)_{14}(AGTG)$ $(GAGT)(GAGA)$	F: TGT CTC TTC GAA ATC AAA CAC AA R: GTT TGA GGC TTA TCT GCC CCT CTC	57	239–303	235	17	0.914	0.908
La45a	$(TCCA)_{15}$	F: AAC CAC ATC TGG CTC AAT CA R: GTT TAG CCC CAG AGT AGG GTG AGA	62	194–254	238	15	0.880	0.871
La49a	$(TATC)_{12}(CATC)(TATC)$	F: GCT GAG GCA GAA ATC ACA C R: GTT ATG TCC ACT GAT GCC TCA AAA	65	224–312	235	19	0.905	0.840
LaC-16	$(TC)_{11}(AC)_8$	F: GGT GTT GAT TGG TCC TCT GG R: GTT TGG GGT TGG TAT TCA TCC AGT	66	146–164	238	10	0.853	0.834

Dive software (Meirmans, 2006). Finally, genetic chord distance, D_{CE} (Cavalli-Sforza and Edwards, 1967), between each population pair was calculated in GENETIX with permutation tests (1000 randomizations) used to estimate *P*-values.

Individual-based analyses may be more suited to questions of dispersal and connectivity because in these analyses, information contained in each individual multilocus genotype is used. By comparison, in population-based analyses, allele frequencies and heterozygosities are calculated for each population. Three different individual-based analyses were employed in this study.

The frequency-based assignment method of Paetkau et al. (1995) was implemented in GENECLASS software (Piry et al., 2004). Populations were determined *a priori* by sampling locations, and GENECLASS generated allele frequencies for each population, excluding the individual to be assigned in the given procedure (Waser and Strobeck, 1998). The expected frequency of each individual's genotype at each locus across all populations was calculated and each individual was assigned to the population from which its multilocus genotype most likely originated. Alleles that were absent from a population were designated a frequency of 0.001.

Genotypes were also analyzed by a Bayesian procedure implemented in the program STRUCTURE (Pritchard et al., 2000). STRUCTURE uses a Markov Chain Monte Carlo (MCMC) algorithm to cluster individuals into populations that each exhibit Hardy-Weinberg and linkage equilibrium (HWLE), without prior definition of the number or geographic location of these populations. Five runs were performed at each value of *K* genetic clusters, with *K* varied from 1 to 5, to ensure proper mixing in the MCMC chain of iterations and consistent results. For all runs we used a burn-in period of 10^6 iterations and followed it by 10^6 MCMC iterations. We assumed an admixture model, in which individuals may have mixed ancestry and correlated allele frequencies, which could account for similarity between closely related populations.

As a third method for inferring genetic structure in *L. analis*, we also used the landscape genetics program Geneland, available in the R statistical package (Guillot et al., 2005a, 2005b). This software operates like STRUCTURE in using Bayesian inference of Mendelian populations in HWLE. But unlike STRUCTURE, Geneland incorporates geographic coordinates of the samples into the prior parameters of the estimation procedure. Recent applications (e.g., Galarza et al., 2009) show promise for inferring structure at low levels of genetic differentiation between marine populations. For spatial coordinates, we ran separate analyses with and without a variable "uncertainty" factor—roughly interpretable as encompassing the home range of an individual fish and appropriate in the case of highly mobile animals (Guillot et al., 2005a). Each run comprised 10^5 MCMC iterations with a thinning set at 100 and *K* genetic clusters varying from 1 to 10; Dirichlet (uncorrelated) allele frequency distributions were assumed and null allele frequencies were explicitly considered (Guillot et al., 2008a, 2008b). Ten independent runs under each set of conditions were launched to check for convergence on *K* populations. Once a reliable estimate of *K* was found, a run with this value fixed was used to estimate and map posterior probabilities of population membership.

Results

High levels of polymorphism were observed in all five populations of mutton snapper at the eight microsatellite loci. The number of alleles detected per locus ranged from nine to 32, and expected and observed heterozygosities ranged from 0.771 to 0.968 and 0.500 to 0.982, respectively (Table 2). Predictably, populations with larger sample sizes exhibited slightly increased levels of allelic diversity; there were 149 alleles present in the JP population and only 135 in the DT population. Also, only two private alleles (i.e. alleles present in only a single population) were present in the DT population, whereas all other populations contained seven or eight. However, there were no apparent trends towards reduced heterozygosity in populations with smaller sample sizes, and estimations of allelic richness indicated that no single population was particularly deficient in genetic diversity across loci.

Seven out of 40 tests indicated significant departures from HWE ($0<P<0.05$). These significant tests were distributed evenly across populations, yet four of the significant tests involved locus La39 (Table 2). After the implementation of sequential Bonferroni corrections (Rice, 1989), only three tests, those involving La39, remained significant. Pairwise locus-population tests of linkage disequilibrium yielded six out of 140 significant comparisons ($0.01<P<0.05$); however, no test remained significant after sequential Bonferroni corrections. Three out of 80 tests indicated significant heterogeneity in allelic distribution between population pairs ($0.01<P<0.05$). Tests of genotypic distributions between population pairs indicated significant heterogeneity in two out of 80 tests ($0.01<P<0.05$). After sequential Bonferroni corrections, no test of genic or genotypic differentiation remained significant.

Values of F_{ST} can range from zero to one with zero indicating the absence of population substructure; values for estimators of this parameter can also be negative, indicating greater heterozygosity within than between populations. In this study estimates of F_{ST} (θ) for each locus ranged from −0.005 to 0 and estimates of R_{ST} (ρ) ranged from −0.009 to 0.005. Pairwise estimates of F_{ST} (θ) ranged from −0.0035 to 0.0022 and pairwise genetic distances, D_{CE}, ranged from 0.012 to 0.018; none of these pairwise comparisons were significant. To address the effect of high variation on estimates of F_{ST}, Meirmans (2006) has developed, ϕ_{ST}, a standardized measure of genetic variation based on the analysis of molecular variance (AMOVA) framework. As with F_{ST} (θ), all pairwise estimates of ϕ_{ST} were negative, ranging from −0.033 to −0.059.

Table 2

Genetic diversity at eight microsatellite loci in five sampled populations of mutton snapper (*Lutjanus analis*). n = number of genotyped individuals; A = number of alleles; $\hat{A}$ = allelic richness; a = number of private alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for Hardy-Weinberg equilibrium: * $P<0.05$, ** $P<0.01$, *** $P\leq0.001$.

	Locus								
Population	La25	La39	La18a	La27a	La34a	La45a	La49a	LaC-16	Overall
Belize (BZ)									
n=49									
A	21	11	31	25	14	13	14	10	139
$\hat{A}$	18.9	9.6	27.0	22.3	13.4	11.5	13.0	9.4	125.1
A	0	0	3	2	0	0	2	0	7
H_E	0.943	0.806	0.968	0.945	0.910	0.856	0.911	0.864	
H_O	0.939	0.750	0.957	0.872*	0.875	0.898	0.896	0.896*	
Honduras (HN)									
n=53									
A	27	13	28	26	17	14	12	10	
$\hat{A}$	21.5	11.3	24.5	22.3	15.6	12.3	11.5	9.5	128.5
a	5	1	0	2	0	0	0	0	8
H_E	0.942	0.813	0.962	0.939	0.926	0.887	0.905	0.847	
H_O	0.868	0.585***	0.962	0.825	0.942	0.865	0.827	0.830	
Puerto Rico (PR)									
n=46									
A	25	12	27	22	16	12	15	10	
$\hat{A}$	21.0	10.6	23.7	21.0	14.9	11.2	13.9	9.3	
a	3	0	0	1	0	1	2	0	7
H_E	0.934	0.771	0.956	0.937	0.911	0.873	0.908	0.846	
H_O	0.957	0.622**	0.957	0.906	0.976	0.884	0.762*	0.841	
Dry Tortugas (DT)									
n=39									
A	24	11	31	19	16	13	12	9	135
$\hat{A}$	21.8	10.3	28.1	19.0	15.4	12.0	11.3	8.7	126.6
a	0	1	0	1	0	0	0	0	2
H_E	0.942	0.794	0.964	0.920	0.915	0.876	0.894	0.859	
H_O	0.895	0.500***	0.947	0.862	0.921	0.821	0.789	0.846	
Jupiter (JP)									
n=55									
A	25	11	32	29	16	12	14	10	149
$\hat{A}$	21.2	9.7	26.8	23.9	14.8	11.4	12.8	9.3	129.9
a	2	0	2	2	0	0	1	0	7
H_E	0.949	0.802	0.966	0.945	0.917	0.891	0.916	0.858	
H_O	0.964	0.537***	0.982	0.841	0.836	0.873	0.891	0.759	

Only 46 individuals (18.8%) were correctly assigned to their source populations with the frequency-based assignment test (Table 3). Individuals were assigned to the population in which their multilocus genotypes exhibited the highest likelihood of occurrence. However, for most individuals, likelihoods of occurrence were similar across populations and success for assignments to origin was similar across populations. Thus, we were unsuccessful at detecting the origin of an individual based on its multilocus genotype and population allele frequencies—not surprising given the similarity in allele frequencies across population samples.

With the Bayesian methods applied in the program STRUCTURE we identified a single genetic cluster with a posterior probability of 1.0. Posterior probabilities were essentially zero for the presence of two, three, four, or five clusters. In the absence of prior information regarding the geographic origin of individuals, we did not detect any genetic structure corresponding to geographic location.

Results from the Geneland analysis broadly agreed with those from STRUCTURE, with subtle differences. Ten independent runs incorporating no uncertainty, or with uncertainty of 120 km^2 in geographic position of

Table 3

Individual assignments based on the frequency test. Values represent the number (%) of individuals from the source populations in each assigned population. BZ = Belize; DT = Dry Tortugas; HN = Honduras; JP = Jupiter; PR = Puerto Rico.

Source population	Assigned population					
	BZ	DT	HN	JP	PR	Total
BZ	11 (22.0)	11 (22.0)	13 (26.0)	11 (22.0)	4 (8.0)	50 (20.4)
DT	7 (17.5)	9 (22.5)	12 (30.0)	8 (20.0)	4 (10.0)	40 (16.3)
HN	11 (20.8)	10 (18.9)	7 (13.2)	15 (28.3)	10 (18.9)	53 (21.6)
JP	10 (18.2)	14 (25.5)	14 (25.5)	11 (20.0)	6 (10.9)	55 (22.4)
PR	9 (19.1)	8 (17.0)	13 (27.7)	9 (19.1)	8 (17.0)	47 (19.2)
Total	48 (19.6)	52 (21.2)	59 (24.1)	54 (22.0)	32 (13.1)	245

individual fish, all converged on a clear mode at K=1, but extensive mixing around this value showed nonzero probabilities of K=2. When geographic uncertainty was increased to 1° on each axis, 3 out of 10 runs converged on K=2. Nevertheless, maps of posterior probabilities from runs with K fixed at 2 (a representative one shown in Fig. 2) showed that the regions encircling our five sampling sites fell into a single genetic cluster at P=1, produced no strong discontinuities in the genetic landscape, and showed that no map regions from which fish were collected grouped with genetic cluster 2 (not shown). Hence the results from both Geneland and STRUCTURE analyses support the existence of a single interbreeding population across the sampled range of *L. analis* (Fig. 2).

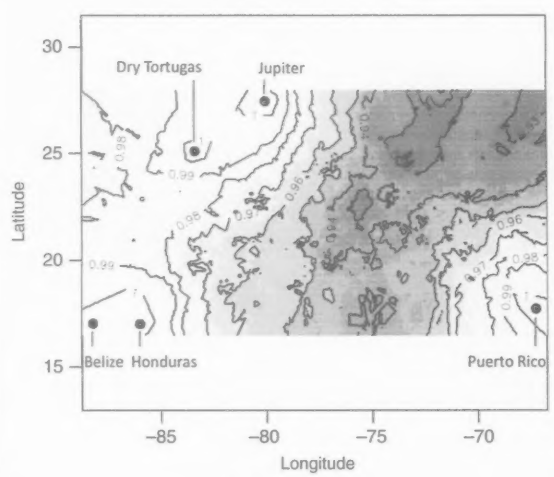

Figure 2

Map of the population membership of mutton snapper genotypes in genetic cluster 1, estimated by the Geneland software package (Guillot et al., 2005b). Contours enclose regions at a given posterior probability of membership. Note that sampled locations all group with genetic cluster 1 at P=1.

Discussion

Based on the results of this study, we cannot reject the null hypothesis that the sampled mutton snapper populations constitute a single panmictic unit. Population genetic substructure is absent from the five sample locations, ranging across approximately 2000 km. Further, because we could not differentiate genetically among the potential sources, it was not possible to ascertain the relative contributions of potential sources to "downstream" populations.

Given these results there are two possible scenarios for the population-level genetic structure. The first is that genetic structure does in fact exist among mutton snapper populations but could not be resolved in this study. If so, future efforts to estimate the relative contributions of mutton snapper spawning aggregations to downstream populations must be explicitly designed to detect such weak structure (e.g., Johansson et al., 2008). This research could require increasing the number of microsatellite loci; in particular, models show that loci exhibiting moderate allelic diversity (i.e., 6 to 10 alleles) are most efficient for population-assignment techniques in weakly differentiated populations (Bernatchez and Duchesne, 2000). Thus, future microsatellite applications to mutton snapper could target these loci in place of the highly polymorphic loci used in our study. In addition, sampling of populations between the relatively distant sites we sampled, as well as outlying populations in the eastern Caribbean, would allow improved evaluation of the genetic landscape of this species.

The geographic scale covered in our study does seem sufficient to recover genetic structure, if it is present. Yellowtail snapper (*Ocyurus chrysurus*) in Belize are genetically differentiated across a

biogeographic boundary from populations along the coast of Brazil (Vasconcellos et al., 2008). Along the Pacific coast of the U.S., copper rockfish (*Sebastes caurinus*) exhibit a pattern of isolation-by-distance over 1400 km, and along the Oregon coast, populations appear to be further structured by habitat and oceanographic barriers (Johansson et al., 2008). Finally, significant genetic structure is present across Barbados, Panama, and Belize in the barred hamlet (*Hypoplectrus puella*) and the black hamlet (*Hypoplectrus nigricans*) (Puebla et al., 2008).

A second, alternative scenario indicates that mutton snapper across the Caribbean region do in fact represent a single panmictic population. Genetic homogeneity across large distances is not an uncommon pattern in marine systems—even with the use high-resolution microsatellite markers. Wreckfish (*Polyprion americanus*) lack genetic structure within both the North Atlantic and South Pacific basins, although interoceanic differences are apparent (Ball et al., 2000). Weak structure across a major biogeographic boundary off the coast of northern Australia indicates high levels of gene flow for the mangrove jack (*Lutjanus argentimaculatus*) (Ovenden and Street, 2003). Heist and Gold (2000) found genetic homogeneity across 1500 km of the Gulf of Mexico for the red snapper (*Lutjanus campechanus*). And bluehead wrasse (*Thalassoma bifasciatum*) lack genetic structure across the entire Caribbean basin (Purcell et al., 2006).

Larval dispersal has long been recognized as a homogenizing force in marine systems. Given a pelagic larval duration (PLD) of 27 to 37 days for mutton snapper, it is reasonable to imagine dispersal by ocean currents across large geographic distances. The PLD of mutton snapper is similar to that of the red snapper (28 to 30 days), a species that exhibits genetic homogeneity across geographic distances comparable to those in the present study (Heist and Gold, 2000). For snapper species the four- to five-week period of time spent in the water column may facilitate long distance dispersal and, thus, genetic homogenization. Although reef fishes shown to exhibit significant population structure tend to have shorter PLDs (beaugregory [*Stegastes leucostictus*]: 19–21 days, slippery dick: 22–30 days, and French grunt: 13–20 days), there are numerous exceptions. Early life history traits, such as PLD, are often unable to satisfactorily explain patterns of genetic structure found across species (e.g., Galarza et al., 2009). Alternatively, the contribution of the larval stage to the maintenance of genetic homogeneity may be due to the interaction of species-specific larval behaviors with the physical environment, rather than a direct function of PLD. The complexity of this interaction could explain the inconsistency in studies that relate early life history traits to broad-scale genetic patterns.

In addition to the pelagic larval stage, dispersal in the adult stage is likely to be a route for gene flow among mutton snapper populations. In fact, annual migrations of tens to hundreds of kilometers to large spawning aggregations may serve to homogenize allele frequencies across a given region (e.g., Florida reef tract) and hence the adult stage may serve to homogenize genotypes at a regional scale, with larval dispersal maintaining genetic connectivity among regions.

Management and conservation implications

In the face of genetic homogeneity across large geographic distances, it is crucial to differentiate between ecological and evolutionary time scales. Boundaries between populations on an ecological scale are not necessarily congruent with those on an evolutionary scale. Our ability to detect ecologically significant population structure with genetic techniques continues to improve, yet remaining challenges limit the inferences we can draw from such a data set. The present study indicates that mutton snapper populations across the study area may be extensively connected over evolutionary time. Yet very little effective migration per generation can prevent genetic divergence among regions (Slatkin, 1987) even while the number of fish arriving from a distant location may be insignificant from the management (i.e., ecological) perspective (Cowen et al., 2007). Thus, ecologically significant dispersal between populations could still be slight, even given the genetic homogeneity observed in the present study. One well-studied example comes from bluehead wrasse, where genetic homogeneity of populations throughout the Caribbean (Purcell et al., 2006) contrasts with results obtained with natural elemental signatures recorded in otoliths (Swearer et al., 1999). Retention signatures found in 89% of summertime recruits to the leeward side of St. Croix indicate high levels of local retention despite the maintenance of genetic homogeneity on a large scale. Thus, future work must continue to try and bridge the distinction between evolutionary and ecological time scales.

The hydrographic environment of the Tortugas South Ecological Reserve, including the formation of the Tortugas Gyre, supports the potential for retention of larvae spawned on Riley's Hump (Lee et al., 1994). Although there is evidence that oceanographic processes in this region can lead to the delivery of these larvae to reefs and nursery habitats of the Florida Keys and southeast Florida shelf (Limouzy-Paris et al., 1997), there is also evidence that some of these same processes can actually advect larvae offshore (D'Alessandro et al., 2007). Thus, at the present time, the pathways of larval dispersal and patterns of connectivity among populations, and the relative contributions of larval sources to fisheries in the Dry Tortugas, Florida Keys, and southeast Florida, remain unclear. Domeier (2004) has provided indirect evidence of a recruitment pathway originating at the TSER that may deliver larvae to the Florida reef tract and to nursery habitats as far north as Palm Coast, Florida. Drifter vials were released over a mutton snapper spawning aggregation site in the TSER for two consecutive years. Based on vial returns, the overall range of dispersal was similar across years, yet the pattern of concentration of returns

was variable. Although drifter vials cannot provide a simulation of larval behavior and only provide beginning and end points in the dispersal process, this study is significant in showing that passive propagules originating in the TSER can in fact be transported to suitable nearshore habitats in a time period similar to average PLDs for reef fishes. This lends support to the idea that genetic homogeneity among mutton snapper populations across the Caribbean does not preclude a substantial contribution of the TSER as a source population for mutton snapper to the Florida Keys and the southeast Florida shelf.

In cases where genetic homogeneity exists in the absence of substantial recruitment from distant populations it will be critical to employ alternative methods to quantify levels and patterns of demographic connectivity among locations. The transgenerational marking of embryonic otoliths with barium stable isotopes is one emerging technique that could directly assign marked larvae and newly recruited juveniles to an adult spawning source (Thorrold et al., 2006). Reef fishes that form large spawning aggregations, such as snapper and grouper, are particularly suited for this application because numerous adults can be marked at once. The barium isotopic signature has been shown to be effectively transmitted to embryonic otoliths in both benthic- and pelagic-spawning fishes (Thorrold et al., 2006). Larvae or juveniles sampled from downstream locations that exhibit this signature in the core of their otoliths can be unequivocally traced back to the spawning source. Although this approach is costly, the information it provides could substantially increase our understanding of demographic connectivity and could be used to gauge the contribution of larval sources.

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Dietary variations in three co-occurring rockfish species off the Pacific Northwest during anomalous oceanographic events in 1998 and 1999

Yong-Woo Lee (contact author)

David B. Sampson

Email address for contact author: Yongwoo.Lee@dfw.wa.gov

Coastal Oregon Marine Experiment Station
and Department of Fisheries and Wildlife
Hatfield Marine Science Center
Oregon State University
Newport, Oregon 97365

Present address for contact author: Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, Washington 98501

Abstract—Stomach samples from three rockfish species, yellowtail (*Sebastes flavidus*), widow (*S. entomelas*), and canary (*S. pinniger*) rockfish, seasonally collected off the Pacific Northwest in 1998 and 1999, provided quantitative information on the food habits of these species during and after the 1997–98 El Niño event. Although euphausiids were the most common major prey of all three predators, gelatinous zooplankton and fishes were the most commonly consumed prey items during some seasonal quarters. The influence of the El Niño event was evident in the diets. Anomalous prey items, including the southern euphausiid species *Nyctiphanes simplex* and juveniles of Pacific whiting (*Merluccius productus*) frequently appeared in the diets in the spring and summer of 1998. The results of stomach contents analyses, based on 905 stomach samples from 49 trawl hauls during seven commercial fishing trips and from 56 stations during research surveys, were consistent with the timing of occurrence and the magnitude of change in biomass of some zooplankton species reported from zooplankton studies in the northern California Current during the 1997–98El Niño. Our findings indicate that the observed variations of prey groups in some rockfish diets may be a function of prey variability related to climate and environment changes.

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Numerous rockfish species inhabit the waters off California, Oregon, and Washington. At least 69 species are found in the northeast Pacific Ocean (Love et al., 2002). Although many of these species have been an important part of the commercially exploited groundfish in this region, rockfish harvests off the U.S. West Coast have been greatly curtailed in recent years because several stocks were assessed as being severely depleted (Parker et al., 2000). Despite the economic importance of these species, we have limited knowledge of their behavior and ecology, including how the individual species interact within the coastal ecosystem. Detailed knowledge about the ecological behavior of fish species, especially their food habits, is essential for developing ecosystem-based approaches to fisheries management because such information will provide the linkages between the fish species in multispecies stock assessment models (Livingston, 1985; Livingston and Jurado-Molina, 2000).

During the unusual oceanographic events of the 1997–98 El Niño and the subsequent cooling phase called La Niña in 1999, we collected information on the food habits of three commercially important rockfish species, yellowtail (*Sebastes flavidus*), widow (*S. entomelas*), and canary (*S. pinniger*) rockfish. Although the diets of these species off the west coast of the United States were examined in two previous studies (Brodeur and Pearcy 1984; Adams 1987), neither study was conducted during anomalous oceanographic events such as El Niño and La Niña years as observed in the current study. Most other studies of rockfish food habits have focused on shallow-water species or have been limited in the geographic and temporal range of their sample collections. The current study not only updates our knowledge of rockfish food habits over wide geographic and temporal ranges but also examines their dietary variations during periods of major environmental change.

Since 1950 there have been seven strong El Niño occurrences (http:\\www.elnino.noaa.gov; accessed July, 2009). The recent event (1997–98) is considered the first or second strongest El Niño in the 20th century, comparable to the record 1983 El Niño (McPhaden, 1999). Peterson et al. (2002) reported changes in the species composition and biomass of the copepod community during El Niño of 1997–98 off central Oregon and found that warm-water copepod species dominated the community during this period. Alternations in the zooplankton community and the unusual occurrence of southern zooplankton species were noted in the northern coastal waters off Oregon and British Columbia during the same period

Table 1

The number of rockfish stomach samples collected from commercial fishing trawlers off the Oregon coast by seasonal quarter and from the NMFS 1998 summer survey cruises off the Oregon and Washington coasts. The numbers of nonempty stomach samples are provided in parentheses.

Sampling dates	Fishing vessel	No. of stations	*Sebastes flavidus*	*Sebastes entomelas*	*Sebastes pinniger*
5–8 Apr 1998	*Pacific*	12	31 (29)	60 (37)	17 (15)
6–9 Aug 1998	*Kelly Girl*	6	31 (29)	56 (48)	29 (24)
8–9 Nov 1998	*Pacific*	7	24 (16)	37 (24)	13 (11)
13–14 Feb 1999	*Pacific*	6	9 (7)	26 (7)	10 (9)
6–7 Apr 1999					
16–18 Apr 1999	*Pacific*	11	54 (45)	48 (46)	27 (6)
13–16 Sep 1999	*Pacific*	7	18 (13)	47 (32)	8 (0)
Subtotal of quarterly fishery sampling		49	167 (139)	274 (194)	104 (65)
27 Jun–7 Aug 1998 (NMFS survey)	*Dominator Vesterallen*	56	360 (312)	—	—

(Mackas and Galbraith, 2002; Keister and Peterson, 2003; Keister et al., 2005). With major changes in environmental conditions, and consequent changes in zooplankton abundance and community structure, one would expect to observe changes in the food habits of planktivorous fish species.

The objectives of this study were 1) to describe the food habits of three commercially important rockfish species (yellowtail [*S. flavidus*], widow [*S. entomelas*], and canary [*S. pinniger*] rockfish) over the study period (1998–99), during which the ocean environment underwent considerable changes, 2) to examine the relationship in these rockfish species between dietary variation and extrinsic factors: spatial (depth, latitude), temporal (time of day, season, year), and biological (predator type, sex, fish size), and 3) to compare the diets of *S. flavidus* collected during El Niño year 1998 with the diets reported during 1980, a non-El Niño year (Brodeur and Pearcy, 1984), to examine possible changes between the two periods.

Materials and methods

Data collection

There were two different types of sampling sources for the study: quarterly fishery samplings and U.S. National Marine Fisheries Service (NMFS) summer surveys. These data sets were maintained and analyzed separately. From the quarterly fishery samplings, stomach samples of three rockfish species, *S. flavidus*, *S. entomelas*, and *S. pinniger*, were collected off the Oregon coast during seven fishing trips aboard Oregon trawlers over six consecutive quarters from spring of 1998 to fall of 1999. Each trip consisted of two to four days of fishing. A total of 398 nonempty stomach samples of the three rockfish species were collected from 49 different stations over the six quarters. From the 1998 NMFS summer bottom trawl survey, stomach samples of only one rockfish species (*S. flavidus*) were collected off the Oregon and Washington coasts, and 312 nonempty stomach samples were collected from 56 stations during this survey (Table 1). Additional data on *S. flavidus* were obtained from stomach contents samples collected off Oregon and Washington during the 1980 summer NMFS survey (R. Brodeur, personal commun.[1]). The data were made available at the individual stomach level (128 nonempty stomach samples) and were compared with the 1998 summer NMFS survey data on *S. flavidus*.

The NMFS samples were collected at stations allocated by a systematic random design. The survey area was divided into three depth strata: continental shelf (55–183 m); shelf break (184–366 m); and uppermost continental slope (366–500 m). Stations were randomly assigned along tracklines laid across the depth strata, with at least one station assigned to each depth stratum along each trackline segment. Each trawl haul was made for 30 minutes at a given station. In contrast, for the quarterly sampling aboard the commercial fishing boats, the fishermen selected the trawling stations on the basis of sonar detection of large schools of rockfishes on the bottom. These different sampling schemes resulted in different coverage of the study area. The quarterly fishery samplings covered a confined area off the Oregon coast, whereas the NMFS survey covered a wider range of coastal waters (Fig. 1), but only during summer.

Individuals of the three targeted rockfish species were randomly selected from the catch and their stomachs were removed at sea. Before a stomach was removed, the buccal cavities were examined for evidence

[1] R. Brodeur. 2001. National Marine Fisheries Service, Northwest Fisheries Science Center, 2030 S. Marine Science Dr., Newport, OR 97365-5296.

of stomach eversion, regurgitation, or feeding in the net. Fish were discarded if they showed signs of any of these actions. Each valid stomach was wrapped in a cloth bag and tied with a tag indicating the species type, sex, fork length (cm), date of sampling, and sampling location, and then preserved in a 10% buffered formalin solution. After arrival at the laboratory, the stomach samples were rinsed with water, and transferred and stored in 70% ethanol for later examination. For prey identification and associated measurements, each stomach was cut open and the contents were blotted dry with absorbent paper. The prey items were examined under a dissecting microscope and identified to the lowest possible taxonomic level. The wet weight of each individual category of prey item was measured to the nearest 0.01 gram and recorded.

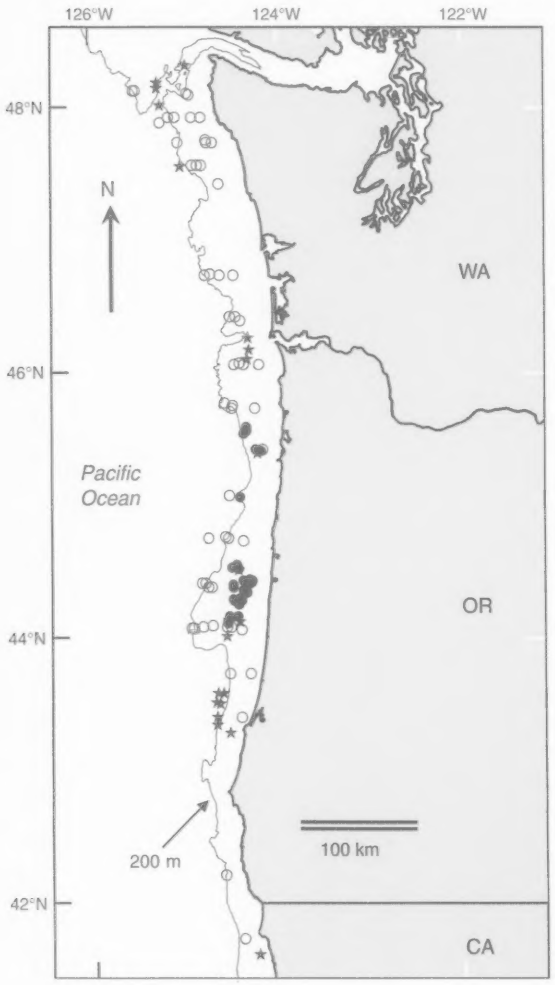

Figure 1

Map of locations sampled for three rockfish species stomachs: *Sebastes flavidus*, *S. entomelas*, and *S. pinniger*. Each symbol represents different sampling collections: solid circles for the seasonal quarterly collections (April 1998 to September 1999); open circles for the 1998 NMFS survey collections; stars for the 1980 NMFS survey collections.

Data analysis

To quantify and summarize the diets within each of the two data sets, the quarterly fishery data and the NMFS summer survey data, we calculated the percent frequency of occurrence and the percent by weight of each prey item for each fish species over each season in a given year. The frequency of occurrence was calculated by dividing the number of stomachs containing a particular prey item by the number of nonempty stomachs in a given season. The percent by weight was calculated by dividing the total weight of a particular prey item by the total weight of the stomach contents in a given season. Samples in the quarterly fishery data set were grouped into four different seasons: winter (December–February); spring (March–May), summer (June–August), and fall (September–November).

Many factors potentially influence the diet patterns of fish, such as biological factors (species, sex, and length), spatial factors (latitude and depth), and temporal factors (daily, quarterly, and annually). Many methods of quantitative analysis, ranging from univariate to multivariate statistical techniques, have been proposed for examining the variation of diets in relation to extrinsic factors (Hyslop, 1980; Cortés, 1997). However, no single method has been accepted as being the best to represent variability in fish food habits. For food-habit studies individual diet information is often aggregated at a population level or across certain extrinsic factors for a statistical comparison of diets. In most studies there is no attempt to formally evaluate diet variability, except at the level of aggregate mean values. Such data aggregation techniques attempt to overcome the multivariate nature of diet data and the problems associated with the high variability and unequal weighting of contents of individual stomach samples. However, data aggregation across a particular factor (e.g., combining samples by latitude class) results in the loss of important diet information at the individual level. It also may result in erroneous conclusions as to the significance of a factor because an analysis based on aggregated data cannot simultaneously account for other factors or assess possible interactions with other factors.

For this study, we employed a multivariate method, principle component analysis (PCA), to examine the patterns of individual diet compositions in relation to sets of extrinsic factors. With the PCA, the measures of diet composition are extracted in a low-dimensional ordination space, based on the individual stomach diet information. We can then relate extrinsic factors to the ordination scores of stomach samples extracted from the PCA to test whether or not the factors are related to dietary variation. We can also gauge the relative

Table 2

Extrinsic factors and their levels that were examined in the analyses of diet variability in rockfish species. Two data matrices were formed for the analyses: quarterly fishery samples (April 1998 to September 1999) and NMFS summer survey samples (1980 and 1998). Given in parentheses are the numbers of nonempty stomach samples for each level of the given factor across all other factors.

Data matrix	Factors	No. of levels	Levels
Quarterly fishery samples	Predator	3	*S. flavidus* (139), *S. entomelas* (194), *S. pinniger* (65)
	Season	6	Six seasons (81, 101, 51, 23, 97, 45)
	Depth	2	<146 m (304), >146 m (94)
	Time of Day	3	Morning (before 10 a. m.) (90) Midday (10 a. m.–5 p. m.) (230) Evening (after 5 p. m.) (78)
	Sex	2	Male (162), Female (236)
	Fish size	3	<40 cm (136), 40–45 cm (203), >45 cm (59)
NMFS summer survey samples	Year	2	1980 (128), 1998 (312)
	Latitude	4	41°–43°5' (51), 43°5'–45° (153) 45°–47° (137), 47°–49° (99)
	Depth	3	<110 m (113) 110–165 m (222) >165 m (105)
	Time of Day	3	Morning (before 10 am) (58) Midday (10 am–5 pm) (260) Evening (after 5 pm) (122)
	Sex	2	Male (241), female (199)
	Fish size	3	<40 cm (125), 40–45 cm (208), >45 cm (107)

importance of the factors by variance partitioning in a linear model.

For the analysis of dietary variation, we formed two matrices of prey species composition data based on the two sampling schemes: a quarterly fishery sample data matrix for the three rockfish species collected from commercial fishing trips, and a summer survey sample data matrix for the *S. flavidus* stomachs collected during the 1980 and 1998 NMFS summer surveys. Some unique extrinsic factors, as well as shared factors, were associated with each data matrix. The unique extrinsic factors associated with the quarterly fishery sample data matrix were predator type and season. We used this data matrix to examine differences among the three rockfish species and the quarterly patterns in their diets but could not explore a latitudinal effect because of the limited geographic coverage of the samples. The unique extrinsic factors associated with the NMFS summer survey data matrix were year (1980 and 1998) and latitude. Other factors tested for both data matrices were depth, time of day, sex, and fish size. All factors were treated as categorical variables. A summary of the factors, their levels, and the number of corresponding stomach samples for each data matrix are given in Table 2.

For the analysis the prey species were grouped into seven major prey groups: euphausiids; fishes; salps; heteropods; jellyfishes (species other than salps and heteropods in the gelatinous zooplankton group); decapods; and miscellaneous prey items. To remove the problem of unequal weights across the samples in the PCA, the weights of prey groups were standardized to proportions based on the total stomach contents weight for each individual fish stomach. In the data matrices each row represented an individual fish stomach and each column represented a prey group. The value of each cell in a data matrix is the weight proportion of a particular prey group in a particular fish stomach. Before running the PCA, we transformed the weight proportions (WP) using the angular transformation, $(2/\pi)$ arcsine $(WP^{1/2})$, which is considered an appropriate transformation for proportion data for improving the assumptions of normality and the homogeneity of variance (Sokal and Rohlf, 1994).

After running the PCA we fitted a series of general linear models (GLMs) to the sample scores extracted from the primary PCA axes to relate the diet compositions to the extrinsic factor. Different strategies are available for the selection of independent variables in a GLM: forward selection; backward elimination; stepwise selection; or use of statistical information criteria (e.g., Akaike information criteria) (Ramsey and Schafer, 2002). For our study, which had limited data, we took a parsimonious approach in selecting variables: a forward selection strategy with a constraint on the maximum level of interactions. We did not consider interactions higher than two-way interactions. Given the limited number of samples (Table 2) it seemed unlikely that

we could detect reliably higher-level interactions. Also, this strategy was necessary because the degrees of freedom could become exhausted as higher interaction terms were added to the model. The model was first fitted with the full set of main effects and the most insignificant terms were then eliminated one at a time until all of the remaining variables were significant ($P<0.05$). The resulting model was then fitted with the selected main effects and all possible two-way interactions of those effects. The model was further refined by removing the most insignificant interaction terms one at a time until no further terms could be removed at a significance level of 0.05.

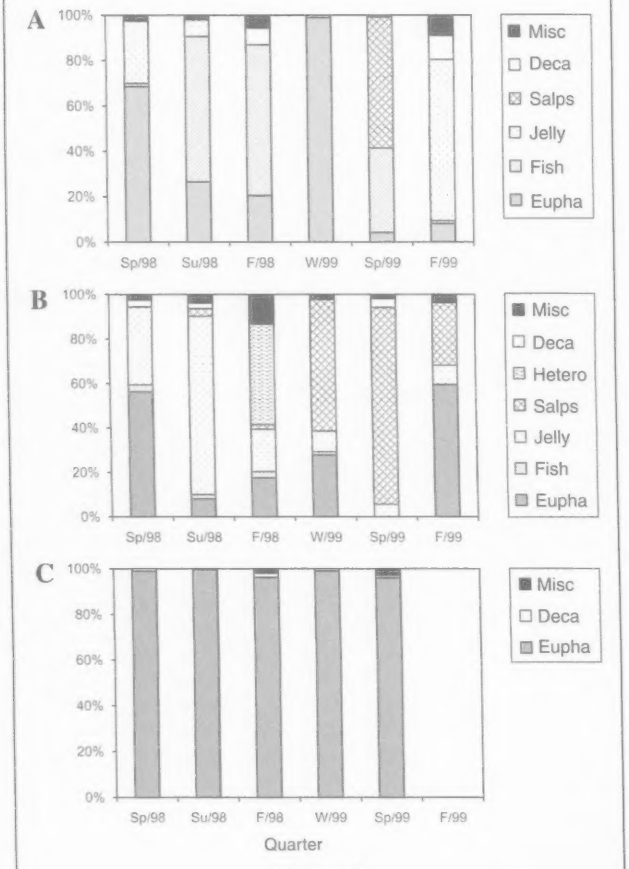

Figure 2

Quarterly variations in stomach contents (percent by weight) of the three rockfish species: (**A**) *Sebastes flavidus*, (**B**) *S. entomelas*, and (**C**) *S. pinniger*. Prey categories contributing more than 1% to the total prey weight in a given quarter were defined as separate prey categories. Trivial prey items, contributing less than 1%, were grouped into the miscellaneous group. The labels for the *x* axis denote the quarter and year: Sp (spring), Su (summer), F (fall), W (winter), 98 (1998), and 99 (1999). Deca=decapods, Eupha=euphausiids, Hetero=heteropods, Jelly=jellyfishes, Misc=miscellaneous.

Results

Description of the diets

The detailed stomach contents data for these species were summarized in terms of frequency of occurrence and percent by weight over each season and year in tables (not presented because of space limitations, but available from the authors). A general description of the diet of each species follows.

***S. flavidus*—Quarterly fishery samples** Yellowtail rockfish preyed upon diverse groups of pelagic planktonic organisms. Although important prey species or groups varied from season to season, in general euphausiids (mainly *Euphausia pacifica*) were the dominant prey group by both occurrence and weight across the seasons (Fig. 2A). Various fish species frequently were found in the diets and fish comprised an important prey group, although the species types varied over the seasons. Juvenile Pacific whiting (*Merluccius productus*) were found in samples from the 1998 spring and summer collections. They were not an important prey item during spring (0.1% by weight, 17.2% by occurrence), but became the most important item by weight in the diets during summer (37.4% in quarterly fishery sampling, 32.5% in the 1998 summer survey). Slender sole (*Lyosetta exilis*) was the top prey item as a single species by weight (42.2%) during the fall of 1998.

Some prey types dominated the diets during certain quarters. Jellyfish species were important in spring and summer of 1998. However, because of their soft fragile body structure and high digestion rate in the stomach, detailed species identification was not possible. Whitish mucus-like digested material was often encountered in the stomachs and was believed to be the digested remains of either Siphonophora or Ctenophora. This unidentified gelatinous zooplankton prey group constituted the number one prey item both by weight (71.1%) and by occurrence (46.2%) in the diets of *S. flavidus* in fall 1999.

Shrimp species were moderately important by weight and occurrence in the fall of 1998 (*Pandalus jordani*, 7.5%) and 1999 (*Sergestes similis*, 9.8%). *Clione* spp. occurred frequently only in the summer 1998 samples from the quarterly fishery and summer survey collections. This prey group was moderately important (10.7% by weight and 27.8% by occurrence) in the 1998 summer survey sample but was less frequent (3.4%) and did not make up more than 0.1% by weight in the quarterly samples. Salps were the most important

prey species both by weight (61.5%) and occurrence (80%) in the spring 1999 samples. The numerous planktonic amphipod species were frequently observed in all seasonal quarters except during the winter of 1999, but there were of minor importance in terms of weight.

S. entomelas—**Quarterly fishery samples** The prey groups eaten by widow rockfish were as varied as those eaten by *S. flavidus*. However, the importance of the fish prey species group in the overall diet was less than it was for *S. flavidus* (Fig. 2B). Juvenile Pacific whiting were found in the diets during the spring and summer of 1998, but were not an important prey in either quarter (<0.7% in weight, <5.4% occurrence). Euphausiids (mainly *E. pacifica* and *Thysanoessa spinifera*) and jellyfishes were major prey items by both weight and occurrence in most quarters. Salps were prey in all seasons and became the most important prey in 1999. In particular, salps were found in more than 95% of the stomach samples collected during the spring of 1999 and were an exceptional prey source in terms of weight (92.6%). This may account for the absence of euphausiid species in the diets during that season. The heteropods was the highest prey group both in terms of weight (45.3%) and frequency of occurrence (45.8%) in the fall of 1998.

S. pinniger—**Quarterly fishery samples** The number of prey taxa identified in the stomachs of *S. pinniger* was substantially fewer than those in the stomachs of S. flavidus and S. entomelas: 22, compared to 45 and 44, respectively. Euphausiid species (mainly *E. pacifica* and *T. spinifera*) were the most dominant (>95%) prey group by weight in the diets over all quarters (Fig. 2C). In some quarters, decapods (shrimps) occurred frequently (around 20%) but with little contribution in weight. Salps did not appear in the diets even during quarters when salps were a major prey item for S. flavidus and *S. entomelas*. Nonempty stomachs were not present in the fall quarter samples of 1999, when few stomachs of *S. pinniger* were collected (n=8).

There were considerable quarterly variations in the prey groups by weight in the diets of *S. flavidus* (Fig. 2A) and *S. entomelas* (Fig. 2B). The dominance and the degree of contribution of the prey groups changed from quarter to quarter for these two fish species. In contrast, over all the quarters studied *S. pinniger* (Fig. 2C) maintained a diet consisting almost completely of euphausiids (>96.1%) but the sample sizes for this species were quite small.

S. flavidus—**NMFS summer surveys in 1980 and 1998** The major differences between the diets of *S. flavidus* in 1980 and 1998 were the occurrence of unusual prey species of southern origin and the dominance of gelatinous zooplankton species in the 1998 samples. The southern prey species were frequently found in 1998 samples, *Nyctiphanes simplex* (euphausiid) and juvenile *M. productus*, with occurrences of 2.2% and 36.8%. These prey species, which are thought to be a major signature of El Niño in the diets of *S. flavidus*, were not found at all in the stomach samples from 1980. *Merluccius productus* was the single most important prey species by weight (32.5%) in 1998, whereas *E. pacifica* was most important (26.4%) in 1980. Fishes were important prey items in 1980. The major species present was herring (*Clupea harengus pallasi*, 18.4%), which was not observed in the 1998 stomach samples. Various gelatinous zooplankton species frequently occurred in the stomach samples, composing the second most important prey group by weight (33.3%) after the fish group (41.4%). The gelatinous zooplankton species found in the 1980 stomach samples were *Sagitta elegans* and *Limacina helicina*, and they made a minimal contribution with less than 0.1% by weight.

Diet comparison based on major prey groups

The weight proportions (average ratio) of the seven prey groups, calculated across the six quarters, demonstrated overall differences in the diets among the three rockfish species during the study period (Fig. 3). Euphausiids were important for all three species, but especially for *S. pinniger* (98.1%). Salps were the most important item for both *S. flavidus* (35.3 %) and *S. entomelas* (49.7%). *S. entomelas* had a tendency to prey more on jellyfishes (25 %), while fishes were a major item for *S. flavidus* (30.2 %).

The major difference between the NMFS summer survey samples for *S. flavidus* in 1998 and 1980 was the role of jellyfishes (22.1%) in 1998 as a major prey group. It was the second most important prey group by weight after the fish group (41.4%) (Fig. 4A). In 1980, jellyfishes did not even represent a major prey group (Fig. 4B). When the 1998 NMFS summer survey samples for the diets of *S. flavidus* are compared with the 1998 summer samples from the quarterly fishery sampling, the more geographically restricted quarterly fishery samples show less feeding on jellyfish (7.3%) (Fig. 2A). The importance of euphausiids by weight was less distinctive in 1998 (20.2%) than in 1980 (47%). It appears that *S. flavidus* depended more on euphausiids in 1980 than in 1998. The importance of the fish prey group in 1998 (41.1 %) was more than the fish prey group in 1980 (32.8%) because of the major contribution of *M. productus* in 1998.

Diet variability in relation to extrinsic factors

The PCA for the quarterly fishery samples was successful in accounting for 73.6% of the total variability in the prey composition data, with the first two PCA axes explaining 43.5% and 30.1%, respectively. Similarly, the percentage of the total variability in the summer survey data accounted for by the first two PCA axes was 66.2%: 41.4% by axis 1 and 24.8% by axis 2. The amounts of variability explained by those PCA axes were sufficient to assure that the PCA components would adequately represent the food habits of the fish species based on individual stomach content information.

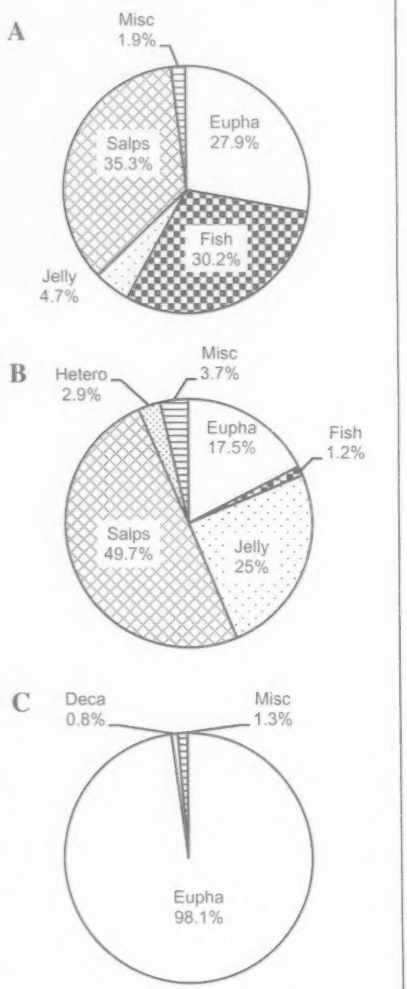

Figure 3

Weight percentages of the major prey groups in the stomach samples of three rockfish species from the quarterly collections (April 1998 to September 1999): (**A**) *Sebastes flavidus*, (**B**) *S. entomelas*, and (**C**) *S. pinniger*. Deca=decapods, Eupha =euphausiids, Hetero=heteropods, Jelly=jellyfishes, Misc=miscellaneous.

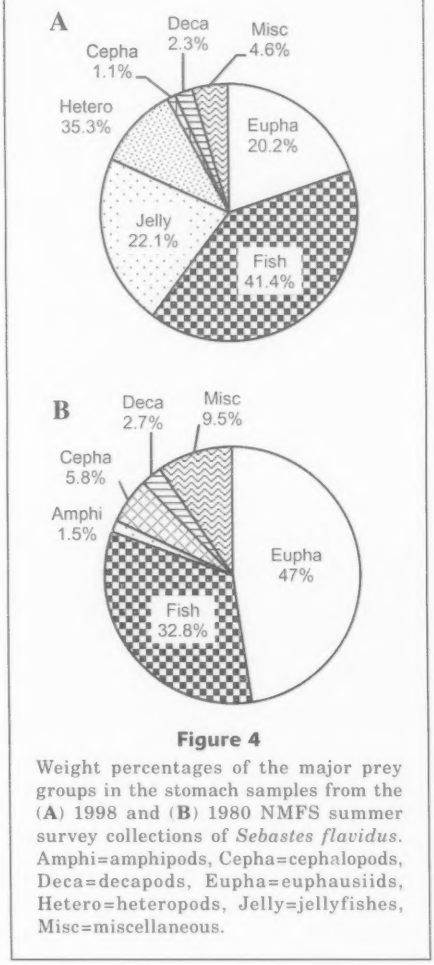

Figure 4

Weight percentages of the major prey groups in the stomach samples from the (**A**) 1998 and (**B**) 1980 NMFS summer survey collections of *Sebastes flavidus*. Amphi=amphipods, Cepha=cephalopods, Deca=decapods, Eupha=euphausiids, Hetero=heteropods, Jelly=jellyfishes, Misc=miscellaneous.

The PCA ordination scores for the individual stomach samples are plotted in the ordination space defined by PCA axis 1 and axis 2 (Fig. 5). The ordination scores of the seven major prey species groups obtained from the transposed PCA analysis are overlaid on the graph. The location of each prey species label on the graph represents the association of that prey species in relation to the surrounding stomach sample points on the graph in terms of its dominance in the diet composition of the stomach samples. For example, the stomach sample points of *S. pinniger* in Figure 5 were tightly clustered around the middle portion of the rightmost side of the graph. When the ordination scores of prey species were overlaid, the score for euphausiids was located very near the stomach samples of *S. pinniger*. This result occurs because the euphausiid group was the dominant prey species group in the diet composition of those stomach samples.

The general linear models for the PCA scores from the quarterly fishery samples were highly significant ($F_{16, 381}$=43.96, P<0.0001, r^2=0.649 for axis 1; $F_{16, 381}$= 30.43, P<0.0001, r^2=0.561 for axis 2), and indicated that the axis 1 and axis 2 scores were significantly related to predator type, season, and their interaction, which implies that the food habits differed between

the species and changed differently over the seasons.

The factor "predator type" accounted for most of the variability in the PCA axis 1 scores in terms of mean squares (MS=10.152, df=2), and season accounted for most of the variability of the axis 2 scores (MS=1.643, df=2). In other words, PCA axis 1 mostly measured differences between the fish species and PCA axis 2 mostly measured quarterly changes in their food habits. In building the models for the food habits of the three rockfish species from the quarterly fishery collections, we found that the other variables considered (depth, time of day, sex, and fish size) were not significant ($P>0.05$). The two sets of PCA scores predicted by the GLMs summarize the diet variation of species over the six quarters (Fig. 6) and show that the diet of *S. pinniger* was stable over the quarters, whereas the diets of *S. flavidus* and *S. entomelas* were not stable and did not vary in parallel.

Separate groupings of the 1980 and 1998 NMFS summer survey samples were fairly evident in the PCA ordination space (Fig. 7), which had a triangular appearance similar to that of the PCA plot from the quarterly fishery collections. Again, the proportions of euphausiids, fishes, and jellyfishes were the primary prey items determining the shape of the PCA plot; the fish stomachs at the points of the triangle had only one prey-item species of euphausiids, fishes, or jellyfishes. However, there were no 1980 summer survey samples in the upper portion of the scatter plot, which corresponded to samples where jellyfishes and heteropods were major prey species in the 1998 samples. This finding, based on individual stomach sample data, corresponds with the result based on the weight proportions calculated from aggregated samples (Fig. 4), in which a dominance of these prey groups in the diets of *S. flavidus* was found in 1998 but not in 1980.

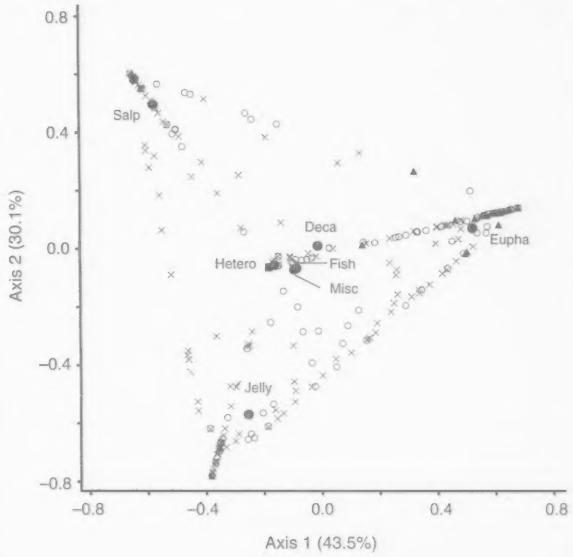

Figure 5

The principle component analysis (PCA) plot for diet compositions of the seasonal quarterly collections of three rockfish stomach species samples: *Sebastes flavidus* (crossed hatch), *S. entomelas* (open circle), and *S. pinniger* (closed triangle). Each point represents an individual stomach sample. In parentheses on the axis labels are the percentages of the total variance in the data explained by each axis. The prey species groups (large closed circles) are overlaid in the same ordination space according to a weighted average of the individual PCA scores: Deca=decapods, Eupha=euphausiids, Hetero=heteropods, Jelly=jellyfishes, Misc=miscellaneous.

According to the general linear model for the PCA scores, the diet composition of *S. flavidus* from the summer survey collections was related with extrinsic factors in a more complicated fashion than was apparent in the quarterly fishery samples ($F_{14, 425}=18.28$, $P<0.0001$, coefficient of determination (r^2)=0.376 for axis 1; $F_{21, 418}=30.43$, $P<0.0001$, $r^2=0.395$ for axis 2). Year, latitude, and depth were significant main effects for the first PCA component scores. The significant interaction terms were year by latitude and depth by latitude. The most influential variable was the interaction between year and latitude (MS=3.714, df=2). The model for the axis-2 scores included terms for time of day and the interaction between time of day and latitude, along with those factors that were significant in the model for axis 1. The interaction between time of day and latitude was the most influential term in the model (MS=1.646, df=5). In general, PCA axis 1 was mainly associated with differences in the food habits between the years and PCA axis 2 with latitudinal effects. The predicted values of the PCA-1 scores for each year and over latitude illustrated that the diets of *S. flavidus* were different in 1998 and 1980, and had different trends with latitude (Fig. 8). Similarly, the predicted PCA-2 scores for time of day and over the latitude showed that the diet pattern differed with the time of feeding and the geographic location of feeding. The complicated models, with significant interactions between temporal and geographical factors, indicate that the *S. flavidus* food habits are not determined by single factors alone. Interestingly, as in the case of the quarterly fishery collections, the fish characteristics (sex and fish size) were not significant explanatory variables for the food habits of *S. flavidus* from the summer survey collection.

Discussion

The three rockfish species examined in this study, which covered a period of unusual oceanographic events (El Niño and La Niña), mostly preyed upon pelagic

macrozooplankton. This finding generally concurred with that from other studies of the diets of rockfish species during normal ocean conditions (Pereyra et al., 1969; Lorz et al., 1983; Brodeur and Pearcy, 1984). However, the dominant prey species differed from previous studies.

The results from the PCA on the diet composition of the rockfish species indicate that patterns in their diets are associated with geographical components (latitude, depth), temporal components (annual, seasonal, diel), and their interactions. The complicated interactions between the geographical and temporal variables in the model for *S. flavidus* from the NMFS summer survey collections, in particular, indicate that the predation pattern for this species was very temporally localized and thus that *S. flavidus* is an opportunistic feeder. An opportunistic feeder in fish ecology refers to a fish species that takes advantage of transitory food sources that are normally outside of its usual diet. The term also describes disproportionately high feeding on food sources that are unusually abundant in a given time frame (Gerking, 1994).

The study by Reilly et al. (1992) on the diets of the pelagic stages of five juvenile rockfish species (*S. flavidus*, *S. entomelas*, *S. goodei*, *S. jordani*, and *S. paucispinis*) off central California found strong annual variation, as well as spatial variation (latitude, depth, and interaction) in the diets, but no significant variation with fish size-class. Even though the life stage of the samples was different from that in our study (adults), there was a strong similarity in terms of significant extrinsic factors related to dietary variability. The opportunistic feeding pattern during the juvenile stage was also consistent with our finding for adult stage of *S. flavidus* and *S. entomelas*. From the finding of high interannual variability in the diet with low intraspecific dietary overlap, they deduced that some pelagic juvenile *Sebastes* spp. had an opportunistic feeding strategy.

One noteworthy finding in our study was that the early juvenile stage of *M. productus* often occurred in the stomachs of *S. flavidus* and *S. entomelas* in the spring and summer of 1998, but then disappeared from both species diets later in the year. Previous studies have not reported Pacific whiting as prey of *S. flavidus* in the northeast Pacific. Pacific whiting are generally known to spawn off southern California during winter (Bailey et al., 1982). However, Phillips et al. (2007) provided evidence that since 2003 there have been northward shifts in the nursery areas of Pacific whiting juveniles into southern Oregon. The appearance of whiting in the diets of *S. flavidus* in our study may be due to the anomalous effects of the 1997–98 El Niño or may reflect a more persistent change.

Another unusual southern species in the diets was the euphausiid species, *N. simplex*. This prey species was commonly observed in the stomach samples of all three rockfish species during the spring of 1998. Brodeur (1986) found this southern species in the stomachs of some fish species off the Pacific Northwest during the 1983 El Niño. Brodeur and Pearcy's (1984), during the non-El Niño year of 1980, did not report *N. simplex* in the diet of either *S. flavidus* or *S. pinniger*. Studies of zooplankton off Oregon and Vancouver Island, farther north of the sampling locations in our study, confirmed the appearance of some southern zooplankton species, including *N. simplex*, during the 1997–98 El Niño years (Mackas and Galbraith 2002; Peterson et al., 2002; Tanasichuk and Cooper 2002; Keister et al., 2005).

Another noteworthy finding of our study was that jellyfish species were the dominant prey for *S. flavidus* and *S. entomelas* in some quarters. The amount and the occurrence of jellyfish species in the diets of *S. flavidus* from the 1998 summer survey collection were much higher than reported by Brodeur and Pearcy (1984). The importance of euphausiids as prey for many planktivorous fish populations, including rockfish species in the northeast Pacific Ocean, has been established in previous studies. However, jellyfish species (gelatinous zooplankton) were ordinarily regarded as minor food sources for most rockfish species. Raskoff (2001) reported detecting changes in the species composition and abundance of jellyfish species off Monterey Bay in California during the El Niño events of 1991–92 and 1997–98. Considering the rapid growth rate

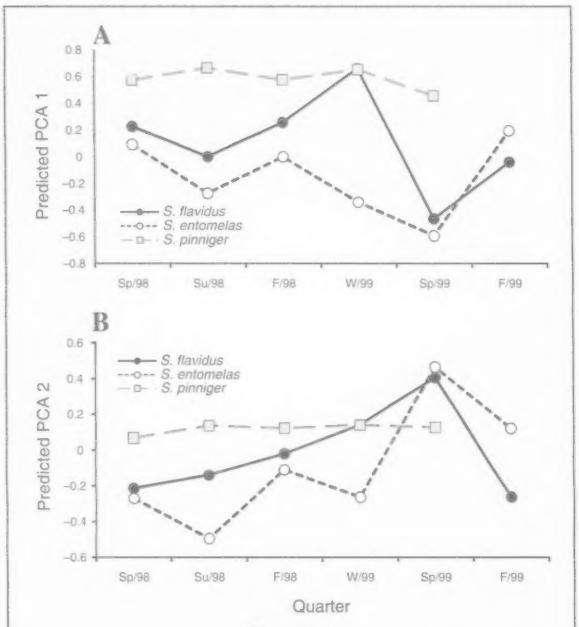

Figure 6

Predicted values by fish species for each seasonal quarter from the general linear model (GLM) fitted to (**A**) the principle component analysis (PCA) axis 1 scores and (**B**) the PCA axis 2 scores from the diet composition data of the quarterly collections.

and turnover times of jellyfishes, it may have been possible for them to become a dominant component of the ecosystem during the period of our study, when it was disturbed first by El Niño and then again by a transition to La Niña (Lavaniegos and Ohman, 2003). In general, quantitative measures of jellyfish in fish diets are likely to be underestimates because jellyfish are rapidly digested and are difficult to identify due to the damage they suffer when consumed and digested (Arai et al., 2003).

Sebastes flavidus and *S. entomelas* exhibited substantial seasonal variations in their diets, as has also been reported in other studies (Brodeur and Pearcy, 1984; Adams, 1987). Interestingly, although the diets of these two species varied from season to season, possibly responding to changes in the prey field, *S. pinniger* continued to prey almost exclusively on euphausiids during all six quarters of our study. This extreme and seasonally constant dominance of euphausiids agrees with the Brodeur and Pearcy (1984) study. Compared with *S. flavidus*, *S. entomelas* seems to have a greater preference for gelatinous prey organisms, such as jellyfishes and salps, which is intriguing because these rockfish species tend to co-occur in the fishery and are considered to occupy the same habitat. The difference between the species does not appear to be an artifact of sampling. For the quarterly fishery collections in 1998 and 1999 all three species were caught concurrently in 15 out of 49 trawl hauls. The proportions by weight of the major prey groups (based on only the data from these 15 hauls) were very similar to the proportions by weight (based on all 49 stations) (Table 3).

Table 3

Percentages by weight of the major prey groups for the rockfish species (*Sebastes* spp.) that collected in the same trawling hauls during the quarterly collections from April 1998 to September 1999. There were 15 hauls out of a total of 49 hauls in which all three rockfish species were collected concurrently.

Prey groups	*S. flavidus*	*S. entomelas*	*S. pinniger*
Euphausiids	25.8	7.8	99.4
Fishes	32.1	0.4	0.1
Jellyfishes	1.4	24.8	0.3
Salps	39.5	63.6	—
Heteropods	<0.1	1.7	—
Decapods	0.6	0.5	0.2
Miscellaneous	0.6	1.2	<0.1
No. of nonempty stomachs	87	104	42

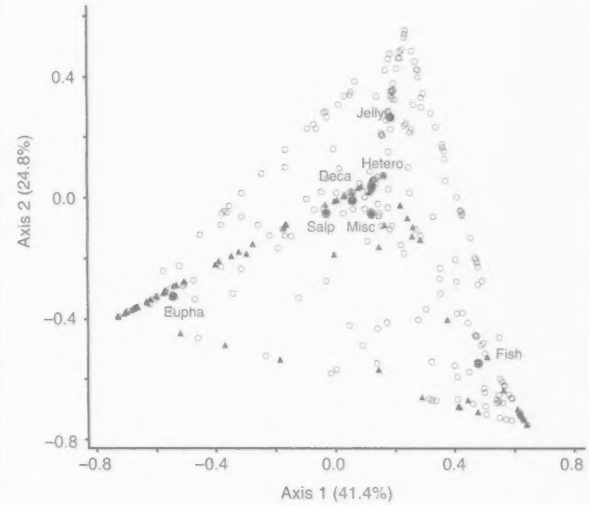

Figure 7

The principle component analysis (PCA) plot of the NMFS summer survey collections: 1980 samples (closed triangle) and 1998 samples (open circles). Each point represents an individual stomach sample. In parentheses on the axis labels are the percentages of the total variance in the data explained by each axis. The prey species groups (large closed circles) are overlaid in the same ordination space according to a weighted average of the individual PCA scores: Deca=decapods, Eupha=euphausiids, Hetero=heteropods, Jelly=jellyfishes, Misc=miscellaneous.

The similarity between the results from the subset and overall data set is important in terms of regurgitation. Although fishes were thoroughly examined for signs of regurgitation, it was still possible that partially regurgitated samples were included as valid intact samples. If that is the case, the data in our study would not provide an unbiased view of the feeding habits of fishes. However, the similarity between the subset and overall set would suggest that the regurgitation issue, even if it existed, did not affect the results of stomach content analyses that were based on proportions. As long as equal proportions of prey items by species and size were lost through regurgitation, then it would not influence the resultant stomach contents analyses. The assumption of loss of equal proportion through regurgitation, however, needs to be validated by alternate methods, possibly by using an underwater trapping device to capture the fish.

The diet analyses in our study indicate that although these three rockfish species may occupy similar geographical habitat, they have evolved to occupy differ-

ent feeding guilds and they differ in their trophic adaptability (Dill, 1983). *Sebastes flavidus* and *S. entomelas* seem to be more opportunistic in their feeding habits and have a greater feeding plasticity in response to changing prey environments, whereas *Sebastes pinniger* is a specialist and has very limited feeding plasticity. The diverse rockfish species complex off Oregon seems to share some common physical features for feeding, but each species still holds distinctive features. *Sebastes flavidus* and *S. entomelas* are characterized as having small mouths with low protrusibility and a relatively long intestine, whereas *S. pinniger* has a high number of long and slender gill rakers that may facilitate its specialized feeding on euphausiids (Pequeño, 1984; York, 2005). Although it is not easy to pinpoint exactly which morphological characteristics are responsible for differences in the diets of the examined rockfish species, the subtle but distinctive dissimilarity in features, which would have been developed through evolutionary adaptations, could in part explain the differences in their diets.

It is challenging to infer which feeding habits are more advantageous to these three rockfish species. But in an environment where food resources are scarce, fish species with specialized feeding habits would generally have a harder time finding adequate prey. It is not known whether euphausiids species are a limiting food resource for these rockfish species or for other fish species in the region. Tanasichuk (1998a, 1998b) noted that euphausiid populations (*E. pacifica* and *T. spinifera*) have decreased fivefold in abundance since the early 1990s near Barkley Sound, a southwest Vancouver Island coastal embayment. It is not clear whether there has been a similar decrease in abundance of the euphausiid populations off Oregon during that time period, but it could be hypothesized that a similar decrease may have caused *S. flavidus* and *S. entomelas* to shift to other available prey resources, whereas *S. pinniger* suffered from the decreased availability of euphausiids. These hypotheses, however, are difficult to test given the lack of information on the trends in macrozooplankton abundance in the area and on rockfish food habits during that time period.

Mackas et al. (2001; 2004) reported observing seasonal and interannual changes in zooplankton biomass and community composition off Oregon and southern Vancouver Island, and reasoned that the changes were responses of the zooplankton community to ocean climate fluctuations and changing current patterns. The waters off California, Oregon, and Washington are subject to broad disturbances by El Niño events, which result in increased surface and near-surface water temperature, elevated coastal sea level, a deeper thermocline, anomalous coastal currents, reduced coastal upwelling, reduced nutrient concentrations and abundance of phytoplankton and zooplankton (Huyer et al., 2002). Keister and Peterson (2003) sampled the zooplankton community off the central Oregon coast over the time period of 1998–2000 and found that there were zonal and seasonal variations in the zooplankton community and that the 1997–98 El Niño played a significant role in structuring the zooplankton community. The effect of the 1997–98 El Niño on zooplankton was noted to have lasted far longer than its physical effects. It is clear from these studies that organisms at lower trophic levels in the coastal ecosystem are strongly influenced by long-term and short-term environmental perturbations. Many rockfish species are bottom oriented but feed on pelagic macrozooplankton species. One would expect these rockfish to be influenced by changes in zooplankton populations or community structure because they feed heavily upon species that are sensitive to environmental changes. However, findings regarding the diets of the three rockfish species in our study indicate that rockfish species do not all respond in the same way to such changes. A given species' response will largely depend upon its evolutionary traits, which govern how it can adapt to changing food environments. It is not known whether strong seasonal variations and the frequent dominance of gelatinous zooplanktons in the diets of *S. flavidus* and *S. entomelas* were just a short-term response to changes in the zooplankton community caused by anomalous oceanic events, El Niño and La Niña, or if these reflect a more

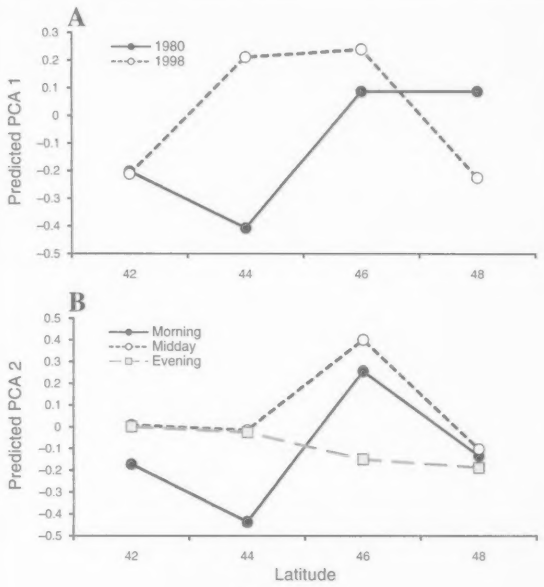

Figure 8

Predicted values from the general linear model (GLM) fitted to the principle component analysis (PCA) axes scores from the *S. flavidus* diet composition data of the NMFS summer survey collections. (**A**) PCA axis 1 predicted values by year for each latitude zone and (**B**) PCA axis-2 predicted values by time of day for each latitude zone.

chronic phenomenon caused by long-term and large-scale climate change.

Although we have attributed the diet variability observed in this study to oceanographic changes, with supporting evidence from other studies, it should be noted that, because of the limited sampling coverage, we could not address the question of how much variability would be due to inherent interannual variability in the diets of these species. Also the prey partitioning and diet variability of these species could be a phenomenon localized only in the study area. Further monitoring of these rockfish species, as well as other fish species in the region, coupled with parallel investigations of the macrozooplankton community would provide a better understanding the relationship between the different trophic levels and the potential consequences of diet changes to the physiology and population biology of these fish species.

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Increases in the relative abundance of mid-trophic level fishes concurrent with declines in apex predators in the subtropical North Pacific, 1996–2006

Jeffrey J. Polovina (contact author)[1]
Melanie Abecassis[2]
Evan A. Howell[1]
Phoebe Woodworth[2]

Email address for contact author: Jeffrey.Polovina@noaa.gov

[1] Pacific Islands Fisheries Science Center
NOAA Fisheries
2570 Dole St
Honolulu, Hawaii 96822-2396

[2] Joint Institute for Marine and Atmospheric Research
University of Hawaii
1000 Pope Rd.
Honolulu, Hawaii 96822

Abstract—Catch rates for the 13 most abundant species caught in the deep-set Hawaii-based longline fishery over the past decade (1996–2006) provide evidence of a change among the top North Pacific subtropical predators. Catch rates for apex predators such as blue shark (*Prionace glauca*), bigeye (*Thunnus obesus*) and albacore (*Thunnus alalunga*) tunas, shortbill spearfish (*Tetrapturus angustirostris*), and striped marlin (*Tetrapturus audax*) declined by 3% to 9% per year and catch rates for four midtrophic species, mahimahi (*Coryphaena hippurus*), sickle pomfret (*Taractichthys steindachneri*), escolar (*Lepidocybium flavobrunneum*), and snake mackerel (*Gempylus serpens*), increased by 6% to 18% per year. The mean trophic level of the catch for these 13 species declined 5%, from 3.85 to 3.66. A shift in the ecosystem to an increase in midtrophic-level, fast-growing and short-lived species is indicated by the decline in apex predators in the catch (from 70% to 40%) and the increase in species with production to biomass values of 1.0 or larger in the catch (from 20% to 40%). This altered ecosystem may exhibit more temporal variation in response to climate variability.

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The North Pacific subtropical gyre is a large oceanic gyre bounded on the south by the North Equatorial Current, on the west by the Kuroshio Current, on the north by the Kuroshio Extension Current and the North Pacific Current, and on the east by the California Current (Pickard and Emery, 1990). Although low in primary productivity, the warm, vertically stratified oligotrophic waters of the subtropical gyre contain a highly diverse food web populated by tunas, sharks, and billfishes at the top trophic levels (Seki and Polovina, 2001; Kitchell et al., 2002). Since the 1950s, the tunas, billfishes, and other large predators in this ecosystem have been targeted by large-scale fisheries. Several studies have indicated possible ecosystem impacts from fishing (Ward and Myers, 2005a; Kitchell et al., 1999; 2002). A comparison of catch, size, and species composition between a research longline survey in the 1950s and observer data from commercial longliners in the 1990s indicated a substantial decline in the abundance of large predators, in the mean size of these predators, and gave some evidence of an increased abundance of formerly rare species (Ward and Myers, 2005a). Models of the North Pacific subtropical gyre were generated with Ecopath with Ecosim, vers. 5 (EwE) modeling software (available from http://www.ecopath.org/index.php) to investigate whether the ecosystem contained any keystone species (Kitchell et al., 1999; 2002). The results indicated that there was not any single species group that functioned as a keystone, but that a broad reduction of apex predators due to fishing might result in an increase in prey (Kitchell et al., 1999; 2002). In effect, the fishing fleet is the keystone predator (Kitchell et al., 1999). However another modeling effort with an EwE model that incorporated some size-class structure revealed that although fishing decreased predator abundance, there was limited evidence (based on the decline in predators) of trophic cascades or other ecosystem impacts (Cox et al., 2002).

The longline logbook and observer data from the Hawaii-based fishery are valuable data sources for investigating the spatial and temporal dynamics of the exploited subtropical ecosystem. The fishery operates over a large portion of the central North Pacific, from the equator to 40°N latitude and from 140°W longitude to the International Date Line. Federally mandated logbooks completed by fishermen provide catch and effort

data on the landed species. A portion of the vessels, randomly selected, also carry observers who record all catches including noncommercial species.

Fishing effort in the Hawaii-based deep-set longline fishery targeting bigeye tuna (*Thunnus obesus*) increased about 250% between 1996 and 2006. The number of fishing sets increased from about 530 per month to 1370 per month, and the number of hooks deployed increased from about 850,000 per month to 2.9 million per month. The catch also increased from 161,000 to 427,000 fishes annually between 1996 and 2006.

In this article, changes in catch rates were investigated within the upper trophic levels of the subtropical ecosystem. Logbook and observer data from the Hawaii-based deep-set longline fishery provided catch and effort data that were used to describe the changes in catch rates of the most commonly caught commercial and noncommercial species from 1996 to 2006. Ecological indicators of the catch were also computed to estimate trends in the exploited ecosystem.

Material and methods

The Hawaii-based longline fishery consists of two components: the daytime deep-set fishery targeting bigeye tuna at depths, and the nighttime shallow-set fishery targeting swordfish (*Xiphias gladius*). The deep-set fishery typically sets hooks between depths of 100 m to 400 m with the median hook depth at about 250 m (Bigelow et al., 2006). Catch data recorded by fishermen in federally mandated logbooks provide daily records of fishing activity such as location, catch by species, number of hooks per set, and since 1996, the number of hooks per float for each set. Deep sets and shallow sets can be identified by a very strong bimodal distribution of the number of hooks between floats. For shallow sets, 2–6 hooks are used per float, whereas for deep sets, 20–32 hooks are used per float (Bigelow et al., 2006). For our analysis we identified deep sets as those with 10 or more hooks per float and shallow sets as those with fewer than 10 hooks per float. The shallow-set fishery operates primarily in the winter and spring within a narrow band of 28–32°N latitude. The shallow-set fishery was closed for several years to reduce interactions with sea turtles. In this article we focus exclusively on the deep-set fishery that operates throughout the year over a broad geographic region and provides an uninterrupted catch and effort time series from 1996. The restriction of our analysis to the deep-set fishery provides a relatively standardized depth range and method of gear deployment. Our analysis was further restricted to data that were obtained from the core region of the fishing ground defined as bounded by 12–27°N latitude. In some years, the fishery made excursions as far south as the equator and as far north as 32°N latitude; however, fishing in these areas was inconsistent over the period of the study.

In addition to logbook records of all commercially valuable catches, a portion of the longline vessels carried observers who recorded all catches and measured a subset of the catches. Between 1996 and 2006 approximately 16% of the deep-set effort in the core fishing ground had observer coverage. The top 13 species in the catch, determined from the observer data, accounted for 90% by number of the total observed catch over the period 1996–2006 in the deep-set fishery in the core fishing ground. In descending order of their proportion in the catch they were bigeye tuna (*Thunnus obesus*), longnose lancetfish (*Alepisaurus ferox*), blue shark (*Prionace glauca*), mahimahi (*Coryphaena hippurus*), sickle pomfret (*Taractichthys steindachneri*), snake mackerel (*Gempylus serpens*), skipjack tuna (*Katsuwonus pelamis*), albacore (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), striped marlin (*Tetrapturus audax*), escolar (*Lepidocybium flavobrunneum*), ono (*Acanthocybium solandri*), and shortbill spearfish (*Tetrapturus angustirostris*). The local name most frequently used in Hawaii for the sickle pomfret is monchong. Other common names used for mahimahi and ono are dolphinfish and wahoo, respectively. Three species reported by observers as part of the catch but not fully reported in logbooks because of their limited commercial value were lancetfish, snake mackerel, and escolar. In recent years escolar has become a commercial species and is now reported in the logbook, but this was not the case in the early part of the time period examined. Escolar is sometimes locally called oilfish or walu, but oilfish is actually the common name for *Ruvettus pretiosus* which represents a relatively rare species in the catch of the longline fishery.

For the 10 species fully reported in the logbooks, we computed a monthly catch-per-unit-of-effort (CPUE) time series. Logbook monthly CPUEs were computed as the total number of fish of a species caught in a month divided by the total number of hooks multiplied by 1000; thus CPUE was computed as the number of fish per 1000 hooks. A generalized additive model (GAM) (Hastie and Tibshirani, 1990) was then used over the 1996–2006 period that fitted observed monthly CPUE and contained a linear function of year to model the time trend, a smoothed monthly term to model the seasonal pattern, and a smoothed spatial term computed from mean monthly latitude and longitude to incorporate any spatial contribution to CPUE.

For the three species not fully reported in the logbooks (lancetfish, snake mackerel, and escolar), we used observer catch and effort data which covered about 16% of the fishing effort over the decade. Because we had much less observer coverage than logbook coverage, we pooled the observer data over the year and computed an annual, rather than monthly, CPUE time series. Observer annual CPUEs were computed as the total number of fish of a species caught in a year on vessels carrying observers divided by the total number of hooks used by those vessels multiplied by 1000. Because of the limited data points with our annual CPUE time series, a simple linear regression was fitted to the annual CPUE data. Although the limited observer coverage was considerably less than that reflected by the logbook data

Table 1

The percentage of the observed catch, the annual percent change in catch per unit of effort (CPUE) from the linear trends, categorical values of production to biomass (P/B), and trophic level for each of the top 13 most abundant species in the observed deep-set longline catch in Hawaii, listed in order of increased annual percent change in CPUE. Trophic level and P/B values are taken from the Ecopath model of Kitchell et al. (2002).

Species	Percentage of total catch		Annual percent change in CPUE	Ratio of production to biomass (P/B)	Trophic level
	1996	2006			
Albacore (*Thunnus alalunga*)	12	2	–9.1	0.6	4.0
Striped marlin (*Tetrapturus audax*)	5	4	–4.8	0.5	4.3
Bigeye tuna (*Thunnus obesus*)	17	17	–3.4	0.8	4.0
Shortbill spearfish (*Tetrapturus angustirostris*)	3	2	–3.3	0.5	4.3
Blue shark (*Prionace glauca*)	12	10	–2.6	0.3	4.0
Skipjack tuna (*Katsuwonus pelamis*)	4	4	0.0	1.9	3.9
Yellowfin tuna (*Thunnus albacares*)	4	4	0.0	1.2	4.0
Ono (*Acanthocbium solandri*)	1	4	0.0	2.0	3.9
Longnose lancetfish (*Alepisaurus ferox*)	10	20	0.0	0.3	3.2
Sickle pomfret (*Taractichthys steindachneri*)	5	9	6.0	1.5	3.2
Mahimahi (*Coryphaena hippurus*)	3	7	6.6	3.0	3.9
Escolar (*Lepidocybium flavobrunneum*)	1	4	10.6	0.8	3.2
Snake mackerel (*Gempylus serpens*)	2	6	17.9	1.0	3.2

for those commercial species where we had both types of data, the observer-based estimates of CPUE compared well with logbook-based estimates. For example, correlations between CPUE trends for commercial species computed from both logbooks and observer data were very similar—0.93 or greater for albacore, striped marlin, shortbill spearfish, bigeye tuna, and sickle pomfret, between 0.80 and 0.89 for mahimahi, ono, and yellowfin tuna, 0.78 for skipjack, and 0.76 for blue shark.

Based on the linear trend derived from either the GAM fitted to monthly CPUE data or the regression line fitted to annual observer CPUE, the annual percent change in CPUE of each species was computed as the slope divided by the intercept multiplied by 12 to convert from monthly to annual values, if necessary, and multiplied by 100 to convert to a percentage. For those species with linear slopes that were not statistically different from zero the annual percent change was set at zero.

From the catch data for the most abundant 13 species, we computed time trends of the annual mean trophic level of the catch, the annual proportion of the catch composed of apex predators (those with trophic level at least 4.0), and the annual proportion of the catch with moderate or high production to biomass (P/B) ratio (defined as at least 1.0). Here we define trophic level 1.0 as primary producers (e.g. phytoplankton), level 2.0 as secondary producers (e.g. zooplankton), level 3.0 as mid-level consumers, and level 4.0 and above as the apex predators. The estimates of trophic level and P/B ratio for most of the 13 species came from a central North Pacific pelagic ecosystem EwE model (Kitchell et al., 2002). The annual trophic level, annual percentage of the catch with trophic level at least 4.0, and annual percentage of catch with P/B ratio of at least 1.0 were computed as a mean weighted by the relative catch in numbers as follows:

$$M_j = \sum_{i=1}^{13} X_i (C_{ij} / CC_j), \quad (1)$$

where M_j = annual trophic level, annual percent of the catch with trophic level at least 4.0, or annual percentage of catch with P/B ratio of at least 1.0 in year j;
X_i = trophic level of species i or binomial variable with value 0 if trophic level < 4.0 or P/B < 1.0 and 100 otherwise;
C_{ij} = catch in number of species i in year j; and
CC_j = combined catch of top 13 species in year j.

Three species—sickle pomfret, escolar, and snake mackerel—are not represented as species groups in the Kitchell et al. (2002) model. However, longnose lancetfish is assigned a trophic level of 3.2 and a P/B ratio of 0.3 in the model. Lancetfish is found from the surface to below 1000 m and feeds on a diverse assemblage of fishes, cephalopods, tunicates, and crustaceans that occupy the scattering layers (Post, 1984). Sickle pomfret, escolar, and snake mackerel all appear to feed on much of the same prey as the longnose lancetfish so it seems reasonable to assign them all to a trophic level of 3.2 (Nakamura and Parin, 1993). For the P/B ratio,

a preliminary growth-rate estimation, based on daily increments on otoliths, indicates that sickle pomfret has rapid growth and reaches 42–49 cm fork length in 12 months (M. Seki, personal commun.[1]).The sickle pomfret growth and maximum size were estimated at just slightly less than the values for skipjack tuna, which has a P/B of 1.9 in the model; hence the sickle pomfret P/B was set at 1.5. Snake mackerel is a relatively fast-growing species with a maximum size of 1.0 m and population doubling time of less than 15 months and therefore it was assigned a P/B of 1.0 whereas escolar has a maximum size of 2 m and is slower growing than snake mackerel, and therefore it was assigned a P/B of 0.8. Recognizing that the P/B values for most of the 13 species are fairly subjective, we used them only to compute the change in the proportion of moderate and high P/B species in the catch where moderate and high P/B species are defined as those with P/B greater than or equal to 1.0.

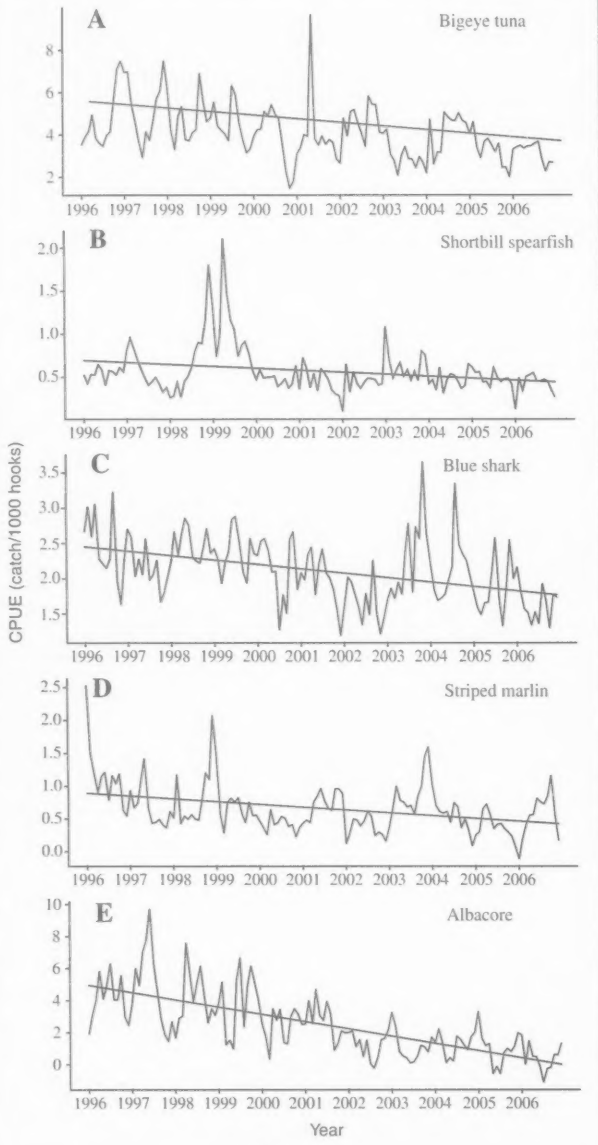

Figure 1

Temporally and spatially adjusted monthly catch per 1000 hooks (CPUE) and linear trend lines from the generalized additive models for those species exhibiting a significantly declining trend in the Hawaii deep-set longline fishery, 1996–2006: (**A**) bigeye tuna (*Thunnus obesus*), (**B**) shortbill spearfish (*Tetrapturus angustirostris*), (**C**) blue shark (*Prionace glauca*), (**D**) striped marlin (*Tetrapturus audax*), (**E**) albacore (*Thunnus alalunga*).

Results

The results from the logbook and observer CPUE time series for the 13 species revealed statistically significant linear trends in slopes ($P<0.05$) for 9 species—5 declining and 4 increasing trends (Table 1, Figs. 1–4). Albacore tuna, bigeye tuna, blue shark, shortbill spearfish, and striped marlin all showed declining CPUE trends; skipjack tuna, yellowfin tuna, ono, and lancetfish showed no significant trends; and mahimahi, sickle pomfret, escolar, and snake mackerel showed increasing trends (Table 1, Figs. 1–4). CPUE trends for albacore tuna, striped marlin, shortbill spearfish, bigeye tuna, and blue shark all decreased from 3% to 9% annually, CPUE trends for yellowfin tuna, skipjack tuna, ono, and lancetfish remained unchanged, whereas CPUE trends for mahimahi, sickle pomfret, escolar, and snake mackerel increased from 6% to 18% annually (Table 1, Fig. 4). For reference, the combined CPUE of all species caught in the deep-set fishery recorded in the observer data declined 4% annually. The species with declining trends had trophic levels of 4.0 or larger and the species with increasing trends had trophic levels of 3.9 or less (Table 1).

The mean annual trophic level of the top 13 species in the catch, weighted by number of fish caught, has declined over the time series by about 0.19 (or 5%), from about 3.85 to 3.66 (Fig. 5). The percentage of the catch of the top 13 species composed of apex predators (trophic level 4 and higher) has declined from about 70% to 40% (Fig. 5). The percentage of the catch of the top 13 species with relatively high P/B,

[1] Seki, Michael P. 2009. Pacific Islands Fisheries Science Center, National Marine Fisheries Service, 2570 Dole Street, Honolulu, HI 96822-2396.

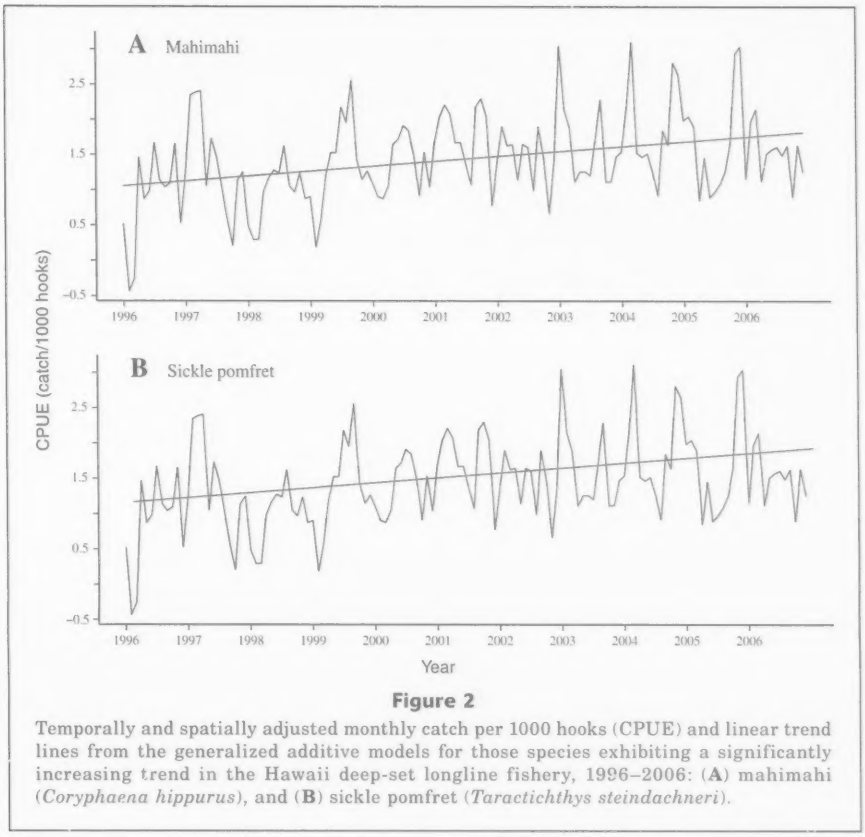

Figure 2

Temporally and spatially adjusted monthly catch per 1000 hooks (CPUE) and linear trend lines from the generalized additive models for those species exhibiting a significantly increasing trend in the Hawaii deep-set longline fishery, 1996–2006: (**A**) mahimahi (*Coryphaena hippurus*), and (**B**) sickle pomfret (*Taractichthys steindachneri*).

greater than or equal 1.0, has approximately doubled from about 20% to 40% (Fig. 5).

Discussion

The longline CPUE, like most fishery-dependent data, responds to a variety of factors that include changes in species targeted, gear changes that impact species catchability, changes in season, and area fished. It is likely that some of these factors have affected the Hawaii-based longline fishery. In an attempt to limit the effect of some of these factors we used only data from the deep-set fishery and from the core geographic region of the fishing ground. Further, for the 10 species for which we had logbook data we used a GAM to account for seasonal and spatial effects. However, in the case of albacore tuna, the 9.1% decline per year may be, at least in part, a result of a shift in targeting. On a basin-wide level the albacore stock, although reduced by fishing, has not exhibited the collapse seen in the deep-set fishery catches (Sibert et al., 2006). Albacore CPUE is greatest in the summer months, and since 2002 a summer fishery for large bigeye tunas has developed at 30°N latitude outside the core area covered in this study. This may have contributed to a shift in targeting from albacore to bigeye tuna.

We observed declines in CPUE trends of large high-trophic-level and lower P/B species, including striped marlin, shortbill spearfish, bigeye tuna, albacore tuna, and blue shark. Increasing CPUE trends were observed for mahimahi, sickle pomfret, escolar, and snake mackerel that are midtrophic-level species with higher P/B values. The increasing trends for mahimahi, escolar, and snake mackerel are most likely not due to increased targeting because snake mackerel has no commercial value, escolar has limited commercial value, and mahimahi is not caught efficiently with deep-set gear (it is generally only caught when the gear is being recovered and hooks are at the surface). The increasing trend is also not likely a response to more hooks being available from the decline in apex species because total catch rates are in the range of 10–20 fish per 1000 hooks and hence hook saturation is not likely a cause. However, the observed increase in catch rates of midtrophic level species concurrent with a decrease in catch rates of apex species is consistent with top-down control where fishing has reduced the abundance of apex species and mid-trophic level species have increased in abundance in response to decreased predation. Mahimahi is an epi-

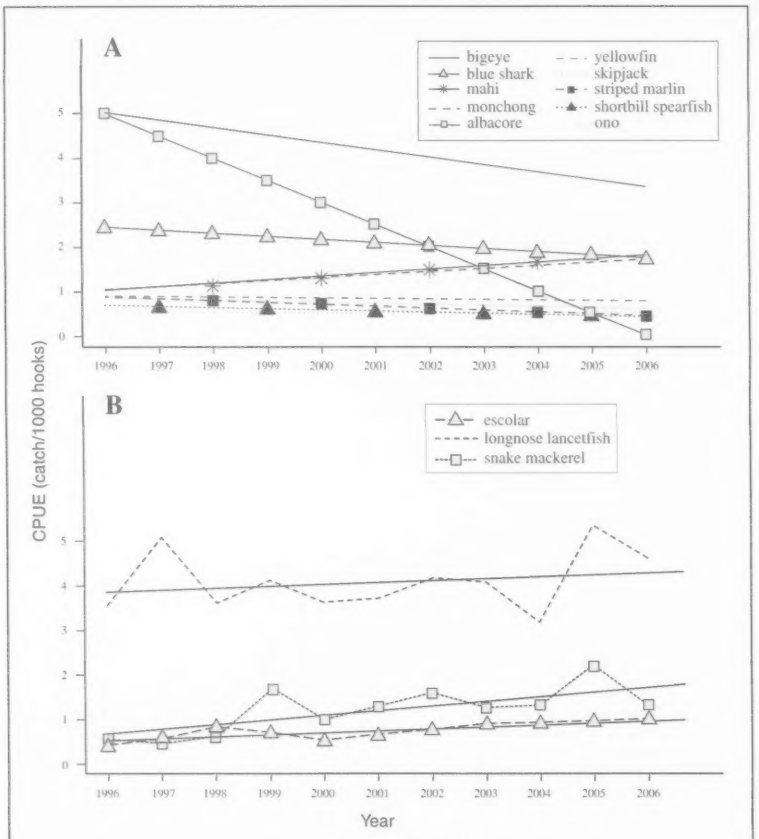

Figure 3

(**A**) Linear catch per 1000 hooks (CPUE) trends for the commercial species in the Hawaii based deep-set longline fishery from the generalized additive models, 1996–2006: bigeye tuna (*Thunnus obesus*), blue shark (*Prionace glauca*), mahimahi (*Coryphaena hippurus*), sickle pomfret (*Taractichthys steindachneri*), skipjack tuna (*Katsuwonus pelamis*), albacore (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), striped marlin (*Tetrapturus audax*), ono (*Acanthocybium solandri*), and shortbill spearfish (*Tetrapturus angustirostris*). (**B**) Annual catch per 1000 hooks (CPUE) and linear regression line for the non-commercial species from the observer catch data in the Hawaii based deep-set longline fishery, 1996–2006: escolar, (*Lepidocybium flavobrunneum*), longnose lancetfish (*Alepisaurus ferox*), and snake mackerel (*Gempylus serpens*).

pelagic species and its predators (billfishes, sharks, and large tunas) have decreased concurrently. An increase in troll and handline CPUE for mahimahi has been observed in the Hawaii fishery since the 1980s (Boggs and Ito, 1993). Sickle pomfret and escolar are mesopelagic species whose predators that include deep-foraging bigeye and albacore tunas, swordfish, and large sharks (Ward and Myers, 2005b). Snake mackerel inhabit both the epipelagic and mesopelagic depths and have many of the same predators as mahimahi in the epipelagic, and sickle pomfret in the mesopelagic depths. We have documented declines in relative abundance of bigeye and albacore tunas, striped marlins, shortbill spearfishes, and blue sharks, all predators of these midtrophic level species. Further, on a Pacific basin scale, biomass of top-level predators, tunas and blue shark, has been estimated to be at 36–91% of the level they would be in the absence of fishing (Sibert et al., 2006).

In a central Pacific EwE model, a top-down control was observed in the simulation (Kitchell et al., 2002; Fig. 3). When an increase in longline fishing was simulated, the biomasses of blue shark, large sharks, brown shark, bigeye tuna, yellowfin tuna, albacore, swordfish, blue marlin, and other marlins all declined; however,

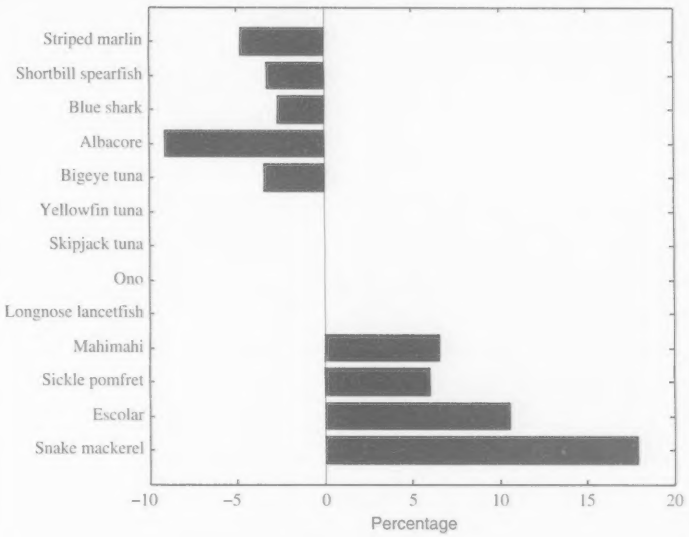

Figure 4

Annual percent change in catch per 1000 hooks (CPUE) (declines in catch are represented by negative values and increases in catch are represented by positive values) from the Hawaii deep-set fishery, over the period 1996–2006, based on the linear trends presented in Table 1 for each species arranged in descending order of its trophic level.

the biomass of mahimahi, flying squid, and lancetfish increased (Kitchell et al., 2002; Fig. 3). The pattern reversed when fishing was eliminated: the biomass of mahimahi, flying squid, and lancetfish all decreased as their predators increased (Kitchell et al., 2002; Fig. 3). Lancetfish CPUE in our analysis showed an increasing trend, but because of its large interannual variation, it was not statistically significant (Fig. 3). Flying squid is not caught in the longline gear. However, as previously discussed, sickle pomfret, escolar, and snake mackerel, although not specifically identified in the Kitchell et al. (2002) model, appear to occupy a very similar prey role in the food web as lancetfish and flying squids. Hence, the observed increase in CPUE for mahimahi, sickle pomfret, escolar, and snake mackerel is consistent with the top-down control seen in the Kitchell et al. (2002) model simulation. Considering an earlier and somewhat different central North Pacific Ecopath model (Kitchell et al., 1999) we concluded that there is no single species that serves as a keystone species in this ecosystem but rather the longline fishery may function as a keystone species.

One additional piece of evidence supporting top-down control for sickle pomfret is that this species was absent in the longline sets of the 1950s but present in the 1990s (Ward and Myers, 2005a). This finding was interpreted as a possible population response to a reduction in predators that included tunas, billfishes and sharks (Ward and Myers, 2005a).

Top-down controls have been observed in temperate ocean ecosystems. A meta-analysis showed shrimp population abundance was controlled by the abundance of its predator, the Atlantic cod (*Gadus morhua*), in eight regions in the North Pacific (Worm and Myers, 2003). Further, at least in one ocean system, the eastern Scotian Shelf, removal of the top predator, the Atlantic cod, resulted in a trophic cascade impacting four trophic levels (Frank et al., 2005). In our pelagic ecosystem, if we considered the longline fleet functioning at the top trophic level, then we have top-down controls spanning three trophic levels: the longline fleet, the apex fishes, and the midtrophic level fishes. Our knowledge of the feeding ecology of many of the midtrophic level fishes that appear to have increased—sickle pomfret, escolar, and snake mackerel—is very limited and therefore we do not know the impacts on the ecosystem from their increased abundance. The juveniles of many of the tunas also occupy the midtrophic level but whether they benefit from the reduction in apex predators or suffer from increased competition or an increase in other predators is unknown but is a critical question for fisheries management. For example, if juvenile tunas are adversely impacted by the increase in other midtrophic level species then a reduction in fishing effort may not result in an increase in adult tunas. Lastly, we do not have data on changes in cetacean abundance and cetaceans have not been included in previous central Pacific models. However, cetaceans are apex predators and if they are

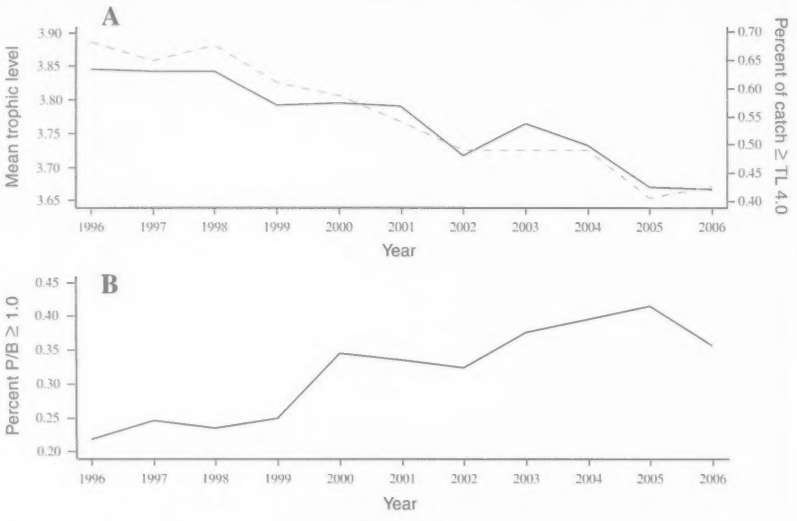

Figure 5

(**A**) Weighted annual mean trophic level of the catch of the 13 most abundant species caught in the deep-set Hawaii-based longline fishery over the period 1996–2006 (solid line), and the percentage of the catch of the 13 species with trophic level (TL) greater than or equal 4.0 (dashed line). (**B**) Percentage of the catch of the 13 species with production to biomass (P/B) greater than or equal 1.0.

not adversely impacted by fishery interactions they may experience less competition because of reduced numbers of apex predator fishes and because more prey would be available with the increase in midtrophic level fishes.

It should be noted that the almost threefold increase in the domestic fishery over the past decade is not the only change in the ecosystem. Over the same period, satellite-derived estimates of surface chlorophyll showed a decline in surface chlorophyll in the Hawaii-based longline fishing ground (Polovina et al., 2008). A change in productivity at the base of the food web could result in bottom-up control that could reduce the abundance of apex predators. Thus it is possible that the substantial changes we have observed in the pelagic ecosystem over the past decade are due to a combination of both bottom-up and top-down controls.

A decline in mean trophic level that exceeds 0.15 has been suggested as representing an ecologically significant fishing down of the food web (Essington et al., 2006). According to this definition the change in the annual mean trophic level of the catch we observed—a decline by 0.19 from 3.85 to 3.66—represents a significant fishing down of the food web in the central North Pacific subtropical gyre. However, an analysis of changes in biomass and trophic level for tunas and blue shark revealed a slight drop in the trophic level of the catch but showed no detectable change in the trophic level of the population (Sibert et al., 2006). A likely reason that we found a more substantial decline in mean trophic level of the catch is that our study encompassed not just the top predators but also midtrophic level fishes.

The decline in the percentage of apex predators from 70% to 40% of the catch and the increase in midtrophic level species from about 20% to 40% of the catch, as well as moderate and high P/B values illustrate the significant increase in the contribution of short-lived, fast-growing, high-fecundity species in the catch and presumably in the exploited population. These species increase their population size rapidly under favorable conditions but given their short life spans, decline quickly in unfavorable conditions. As a result, the current ecosystem will likely exhibit greater temporal variation in response to climate variation.

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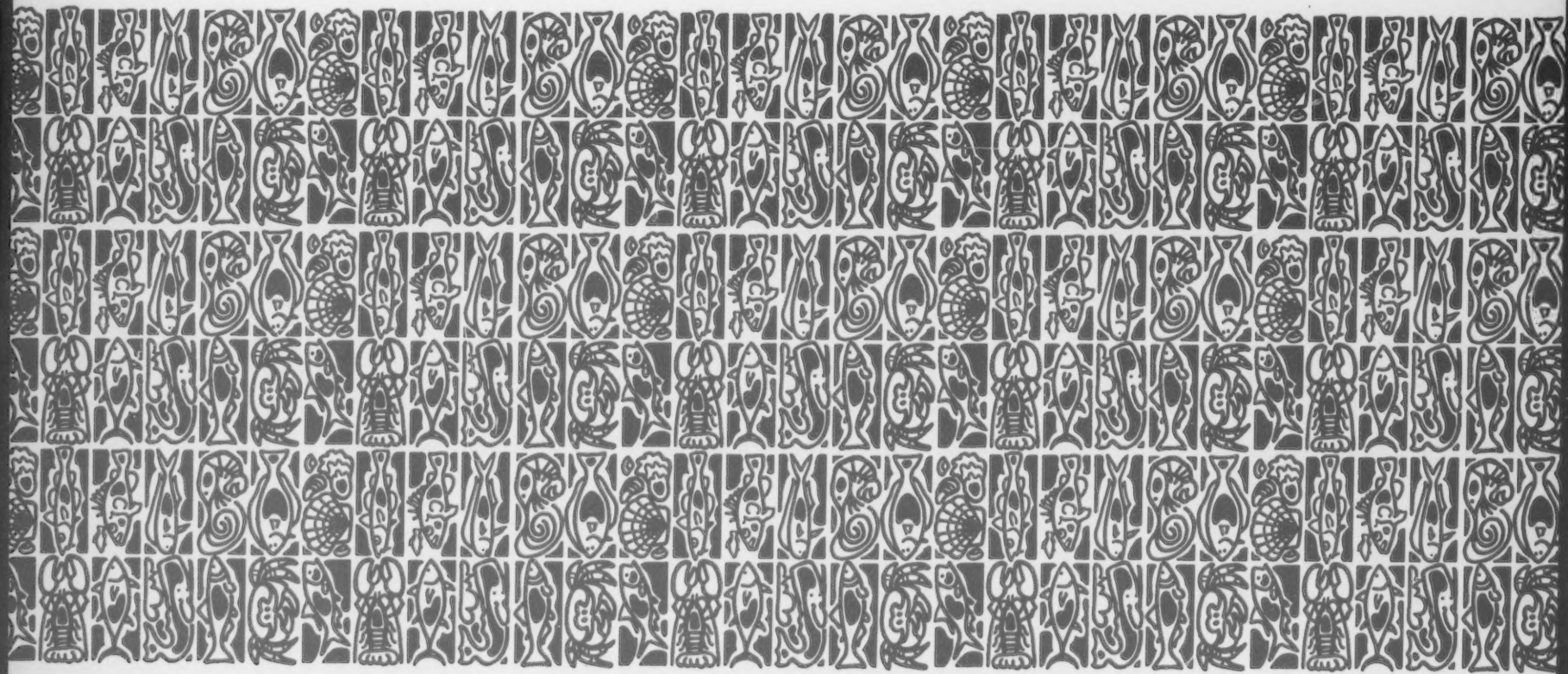
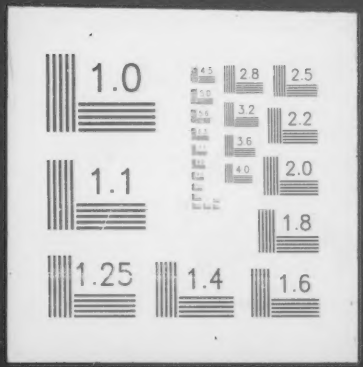
This statement is required by the Act of August 12, 1970, Section 3685, Title 39, U.S. Code, showing ownership, management, and circulation of the *Fishery Bulletin*, publication number 366-370, and was filed on 20 August, 2009. The *Bulletin* is published quarterly (four issues annually) with an annual subscription price of $36.00 (sold by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402). The complete mailing address of the office of publication is NMFS Scientific Publications Office, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115. The complete mailing address of the headquarters of the publishing agency is National Marine Fisheries Service, NOAA, Department of Commerce, 1315 East-West Highway, Silver Spring, MD 20910. The name of the publisher is Willis Hobart and the managing editor is Sharyn Matriotti; their mailing address is: NMFS Scientific Publications Office, 7600 Sand Point Way NE, Seattle, WA 98115. The owner is the U.S. Department of Commerce, 14th St. N.W., Washington, DC 20230; there are no bondholders, mortgages, or other security holders. The purpose, function, and nonprofit status of the organization (agency) and the exempt status for Federal income tax purposes has not changed during the preceding 12 months. The extent and nature of circulation is as follows: total number of copies (A) (average number of copies of each issue during the preceding 12 months) was 1021 and the actual number of copies of the single issue published nearest to the filing dates was 1001. Paid circulation (B) is handled by the U.S. Government Printing Office, Washington, DC 20402, and the total number printed for sales (mail subscriptions and individual sales) was 225 for the average number of copies each issue during the preceding 12 months and 225 the actual number of copies of the single issue published nearest to the filing date (C). Free distribution (D) by mail; samples, complimentary and other free copies (average number of copies each issue during the preceding 12 months) was 756 and the actual number of copies of the single issue published nearest to the filing date was 736. Free distribution outside the mail (E) by carriers or other means was 0 for both average number of copies and actual number of copies. Total free distribution (F) was 0 for both average number of copies and actual number of copies of the single issue published nearest the filing date. The total distribution (G: sum of D and B) (average number of copies each issue during the preceding 12 months) was 981 and the actual number of copies of the single issue published nearest to the filing date was 901. There were 40 copies (avg. annual) not distributed (H). The total (I: sum of G and H) is equal to the net press run figures shown in Item A: 1021 and 1001 copies, respectively. I certify that the statements made by me above are correct and complete: (Signed) Willis Hobart, Publisher.

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